Laboratory of Biosensors and Nanomachines

Università di Roma Tor Vergata

MARIE CURIE ACTIONS

Horizon 2020

# DNA-based nanomachines powered by biological inputs for diagnostic and drug delivery applications


***Simona Ranallo***

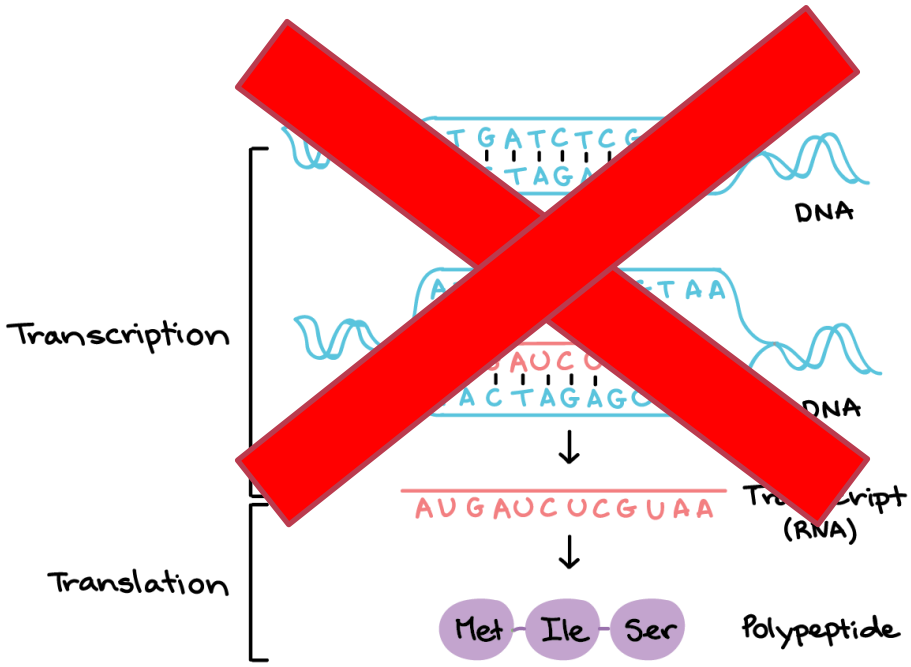
*University of Rome Tor Vergata*
*University of California Santa Barbara (UCSB)*

*simona.ranallo@uniroma2.it*


**University of Teramo 3rd May 2022**

# DNA in Biological Organism

DNA's chief function is to serve as a read-only memory whose information content is transcribed into RNA before this information is used to orchestrate life's functions through the synthesis of protein and RNA-based molecular machines.

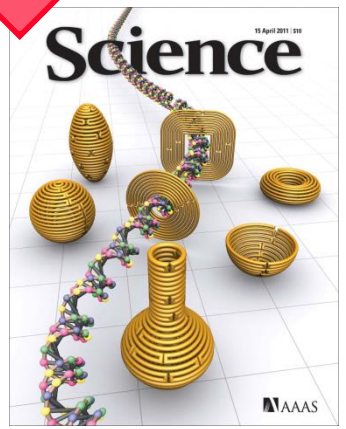


# DNA Nanotechnology


**DNA nanotechnology:**

Design and manufacture of artificial nucleic acid structures for technological uses.

**DNA is used as engineering material**
**NOT as a carrier of the genetic information!**




**A bottom up approach:**

DNA-nanotech uses the internal information of DNA molecules to guide their autonomous self-assembly into nanostructures.


# Why DNA as nanomaterial building block?



- Easy to synthesize (low cost?)
- Easy to engineer to attach molecules
- Biocompatible
- Chemical robustness
- Programmable (predictable base-pairing (A-T, G-C)
- Recognition of different targets
  (DNA strands, protein, etc.)
- The stiffness of duplex DNA enables its assembly
- Difference in stiffness between duplex DNA
  and single-stranded DNA (ssDNA as hinges)
- Relative weakness of interactions



Seeman, N.C. (1982). Nucleic-Acid Junctions and Lattices. Journal of Theoretical Biology 99, 237-247.

# DNA Nanotechnology

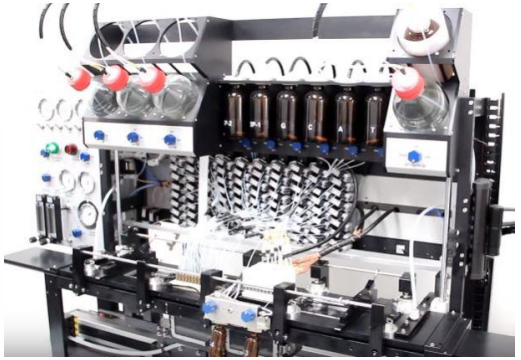
01 Structural DNA Nanotechnology: 2D

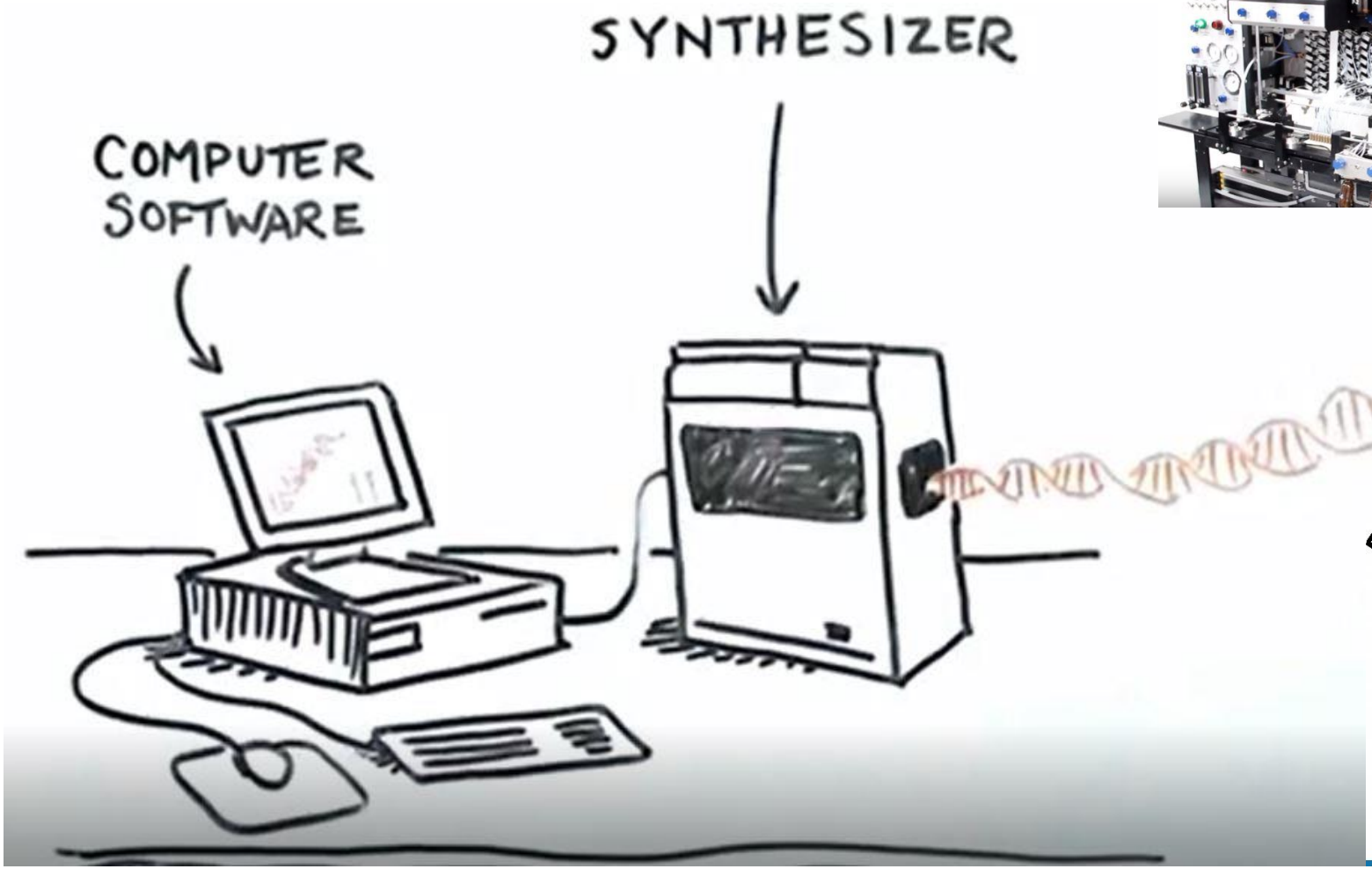
02 Structural DNA Nanotechnology: 3D

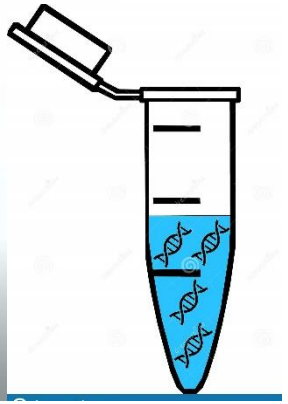
03 Functional DNA Nanotechnology

04 Structural + Functional DNA Nanotechnology

# Solid-phase synthesis of DNA





# Structural DNA-Nanotechnology

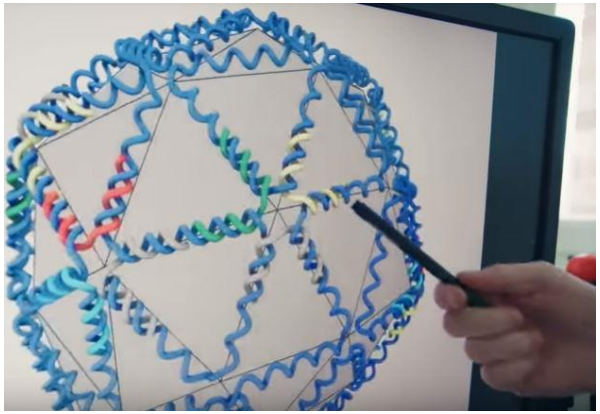
To build object of any shape at the nanometer scale through self assembly of DNA strands.

To use the internal information of oligonucleotide sequences to self-assembly nanostructure

# Structural DNA-Nanotechnology

1. Draw the raw shape of the structure!

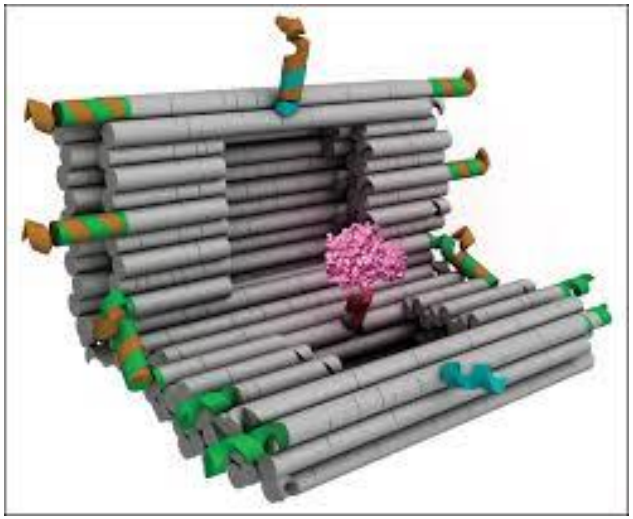
2. An algorithm calculates how the DNA scaffold strand will arrange itself on the surface of the structure and will also calculate all the short DNA strands.



# Structural DNA-Nanotechnology

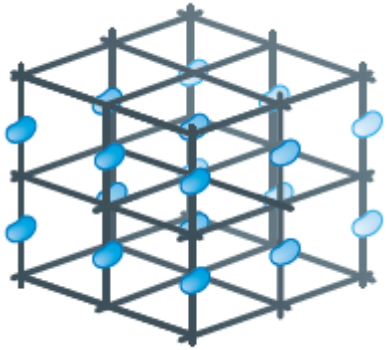
3. Order the oligonucleotides

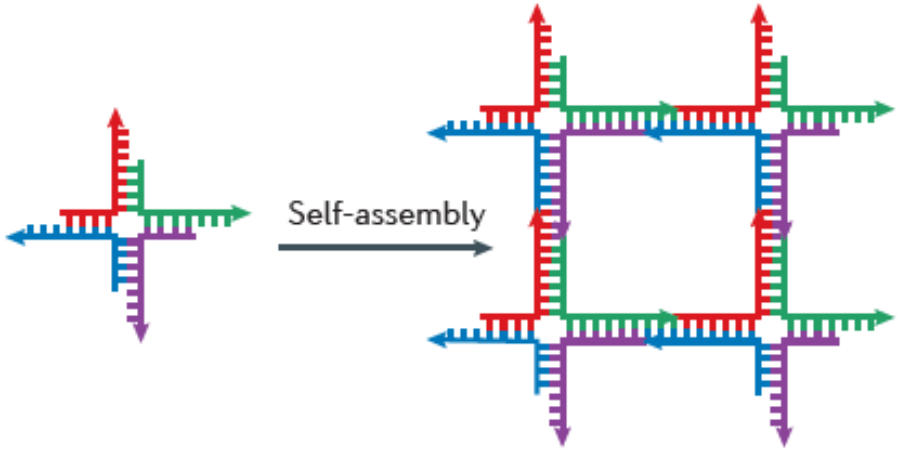
4. Mixing, annealing over night and the DNA nanostructure is done!



# The early years: the Ned Seeman's dream

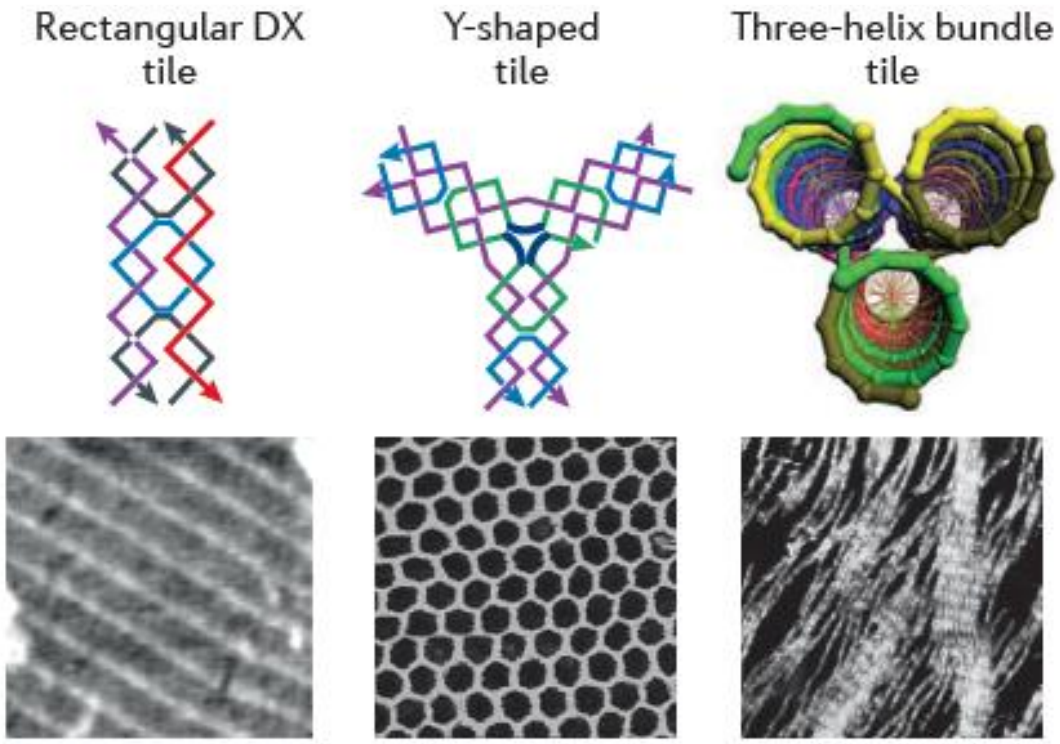
Rather than relying on trial and error to crystallize biological macromolecules, the idea was that crystals could be assembled using WC-interactions






Branched junctions could be connected together by the base pairing of single-stranded overhangs, called sticky ends, into a 3D crystalline material

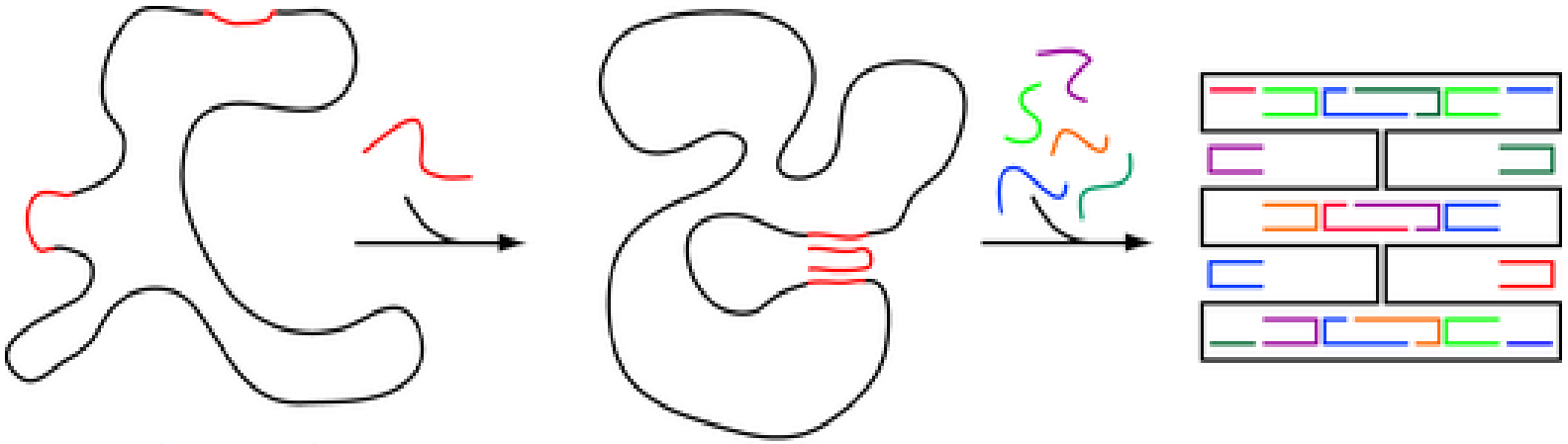
# DNA tile self-assembly: 2D DNA crystals by design

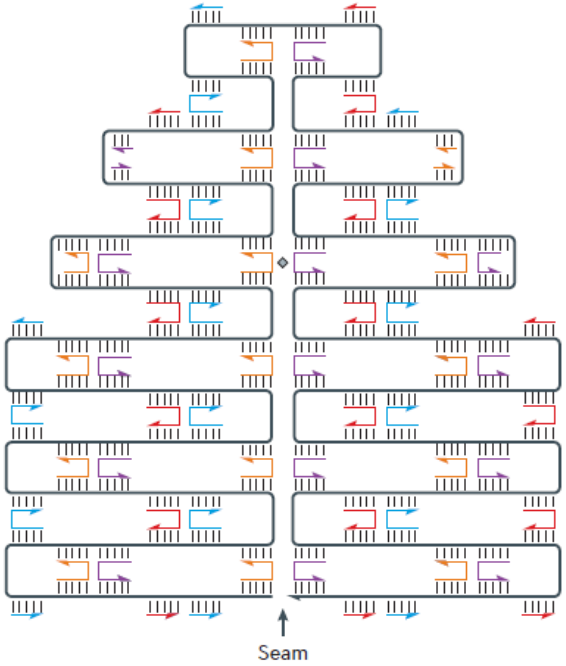


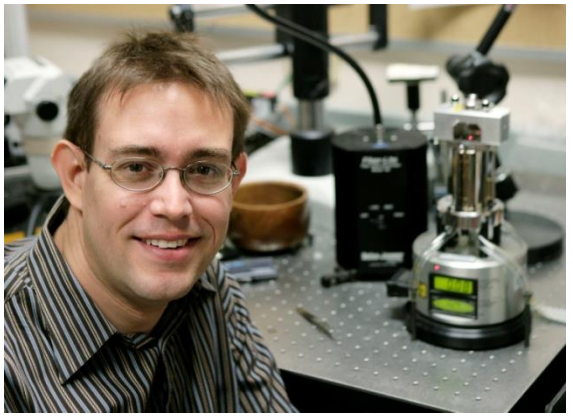

1) Making arms with unique sequences (limited branch migration)
2) Three-arm junctions , and double-crossover (DX) molecules comprising two DNA double helices linked together
(geometric rigidity, proper stability and topology)

Lin, C. et al. DNA tile based self-assembly: building complex nanoarchitectures. *ChemPhysChem* **7**, 1641–1647 (2006).

# DNA Origami



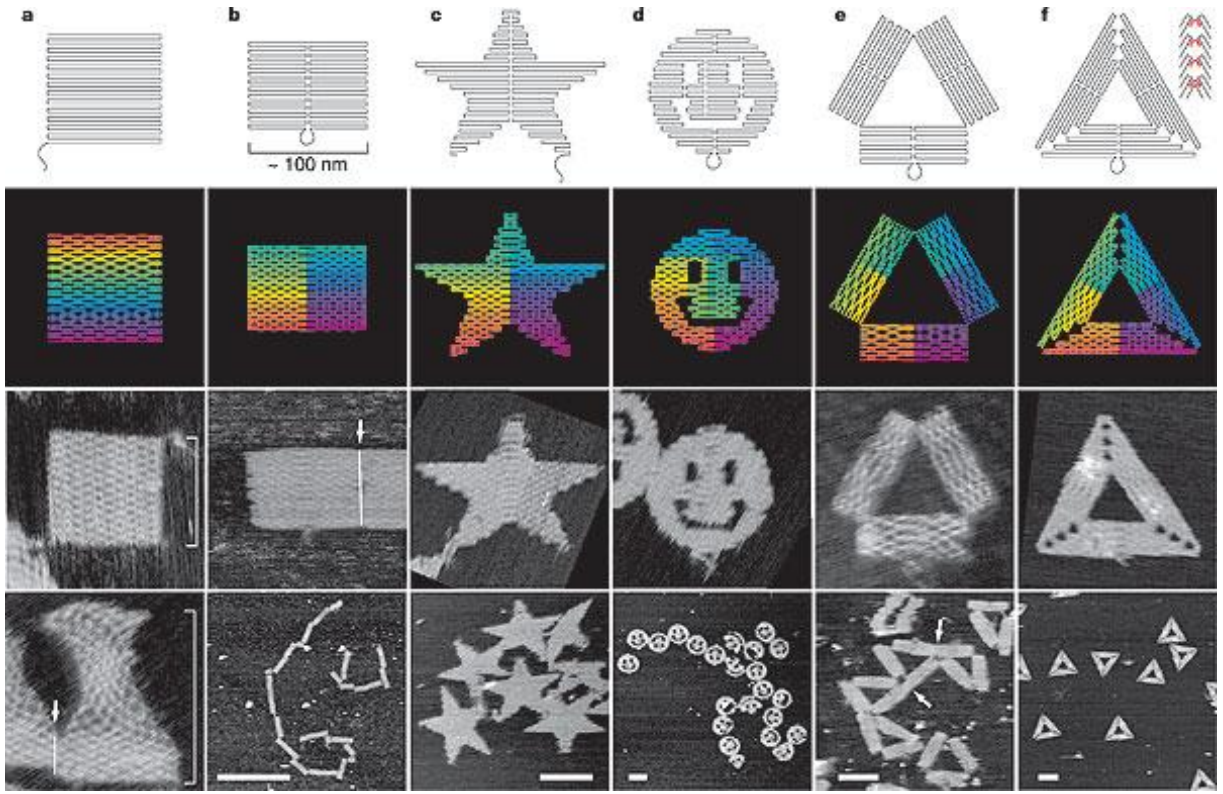


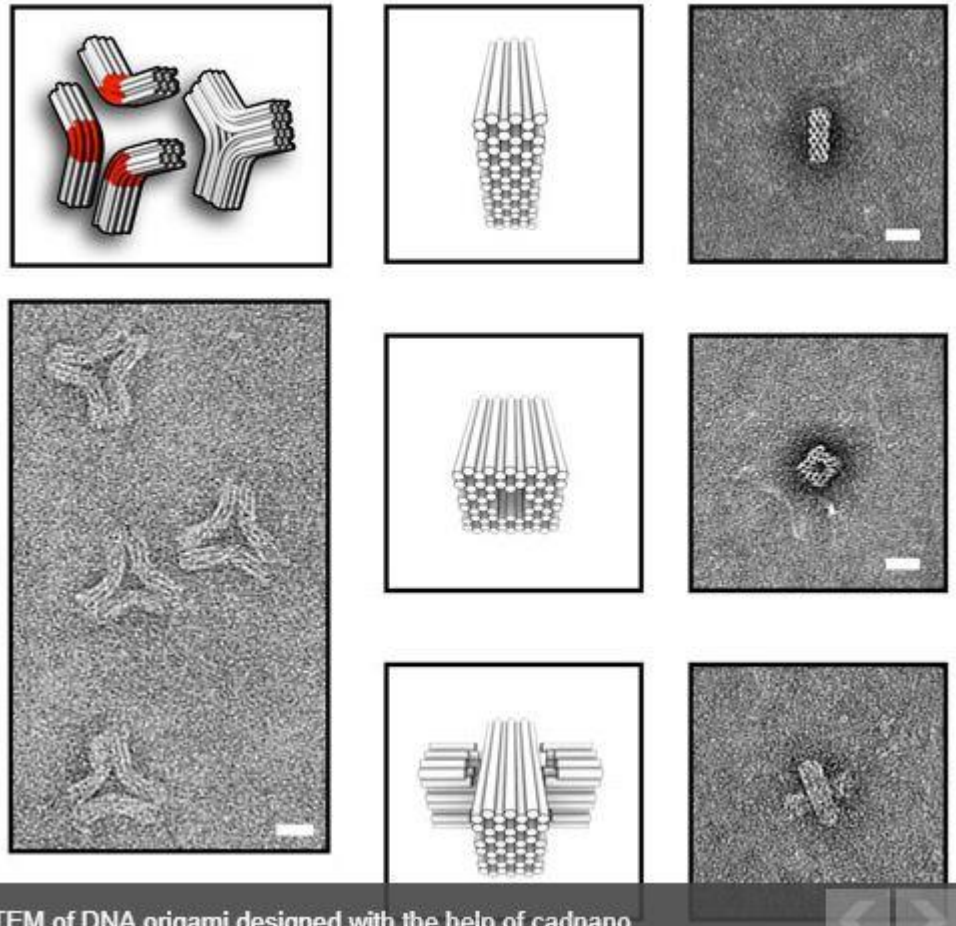
Rothemund, P. W. Folding DNA to create nanoscale shapes and patterns. *Nature* **440**, 297–302 (2006).

# DNA Origami: 2D Structure

Non-periodic 2D structures of arbitrary complexity can be made, such as a map of the Americas, rectangles, smiley faces, stars and other designed patterns.




Rothemund, P. W. Folding DNA to create nanoscale shapes and patterns. *Nature* **440**, 297–302 (2006).



TEM of DNA origami designed with the help of cadnano.

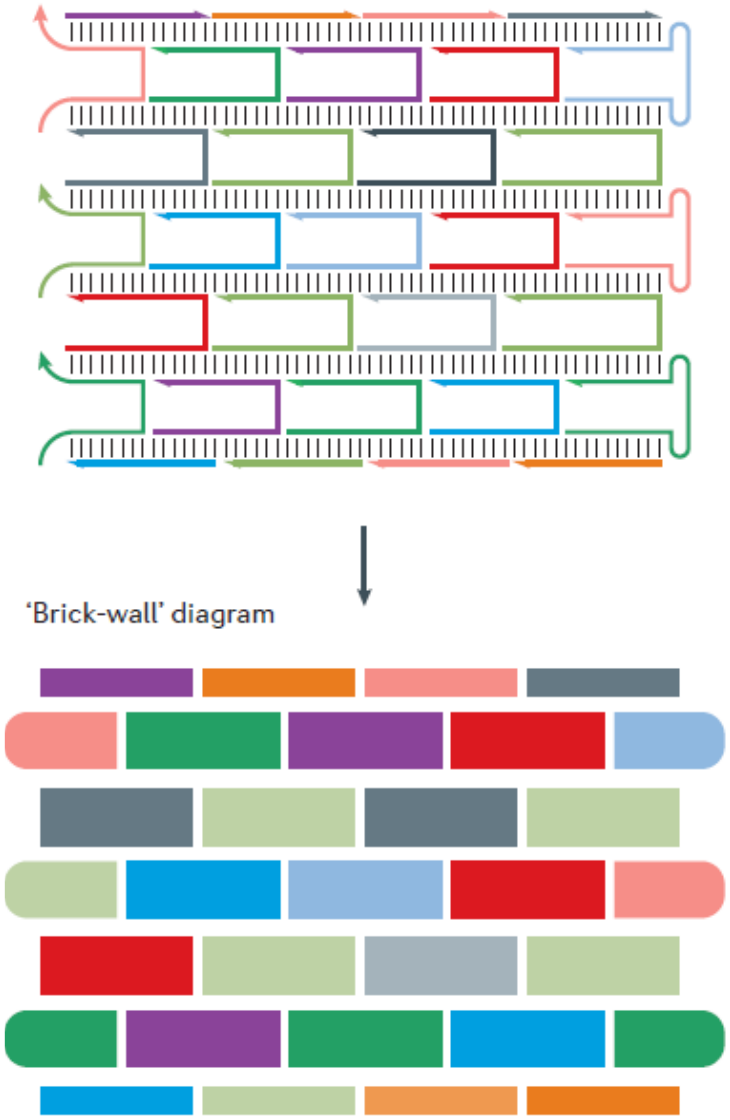
**cadnano** simplifies and enhances the process of designing three-dimensional DNA origami nanostructures. Through its user-friendly 2D and 3D interfaces it accelerates the creation of arbitrary designs. The embedded rules within **cadnano** paired with the finite element analysis performed by cando, provide relative certainty of the stability of the structures.

### cadnano features:

- Platform independent (tested in Windows, OSX and Linux)
- Visual cues aid design process for stable structures
- 3D interface powered by Autodesk Maya*
- Open architecture for plug-in creation
- Free and open source (MIT license)

**DOWNLOAD CADNANO**
*It's free and open source.*

# “Brick assembly”: a new strategy to generate 2D Nanostructures

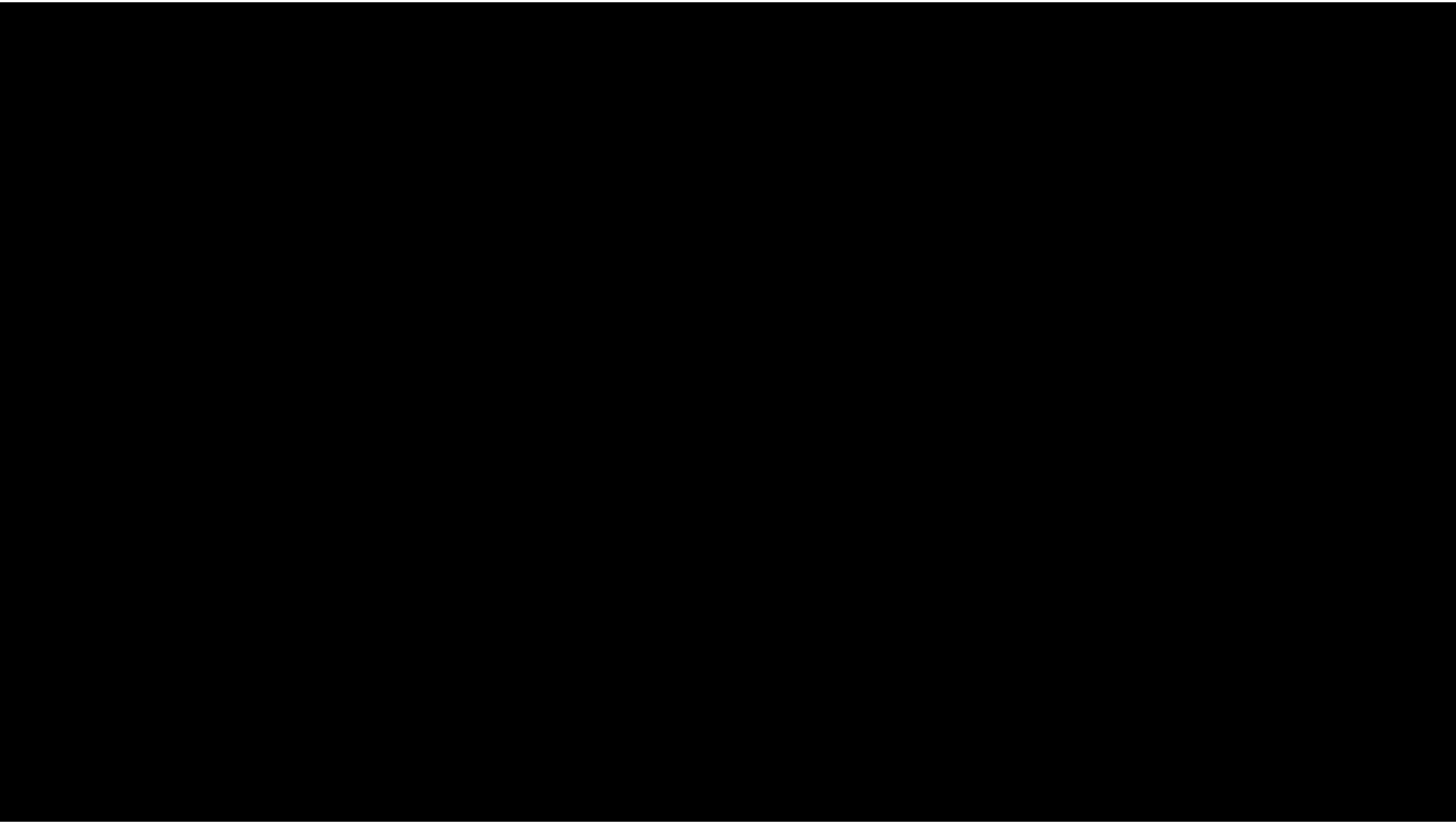



The building blocks are single strands of DNA containing four modular domains, which are designed to form interconnected staggered duplexes with one another, resulting in DNA lattices.

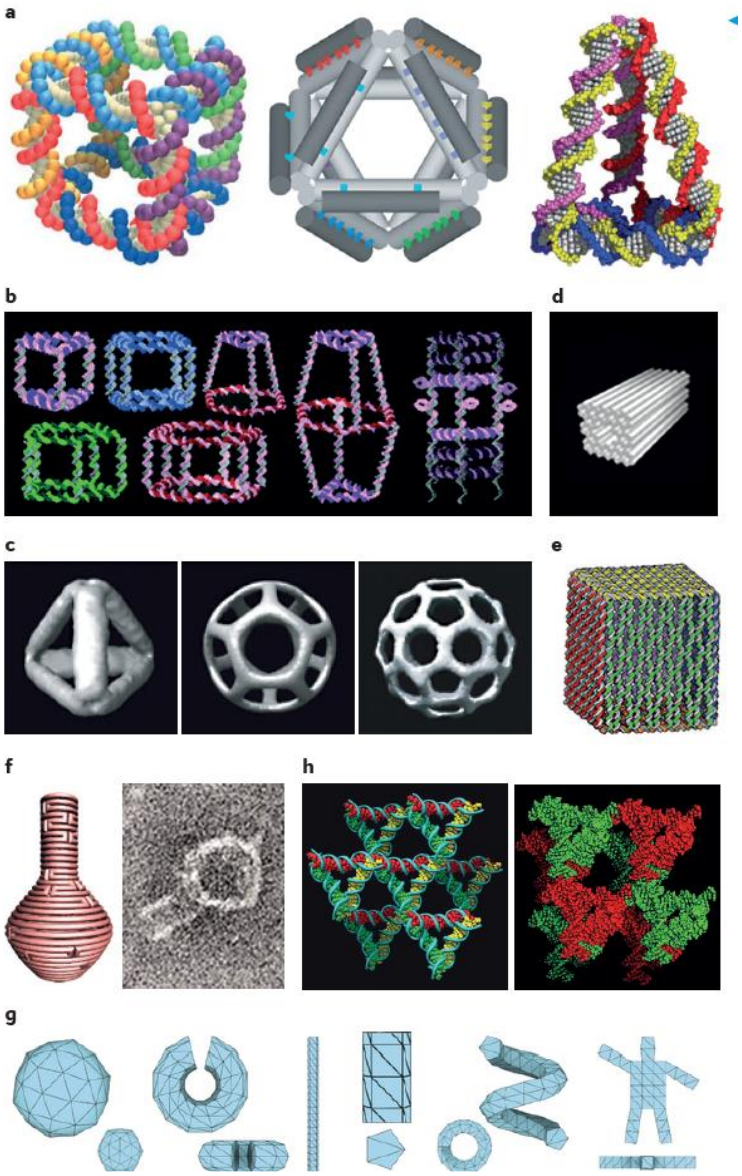
- The sequences are all unique.
- Any arbitrary shape by selecting the set of strands that defines the structure.

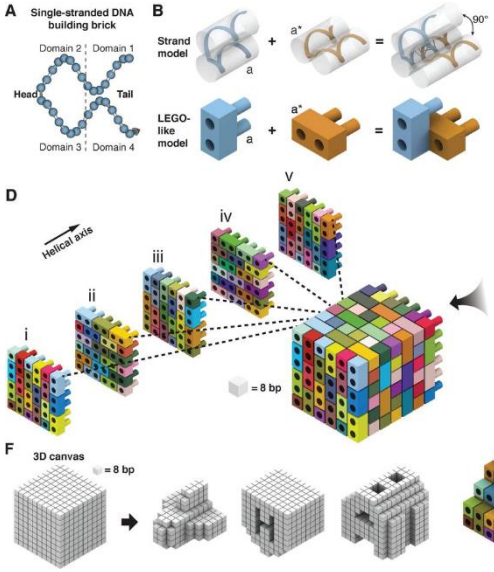
Wei, B. et al. Nature 485, 623–626 (2012).

# Building 3D Structures with DNA Bricks



# 3D Nanostructure



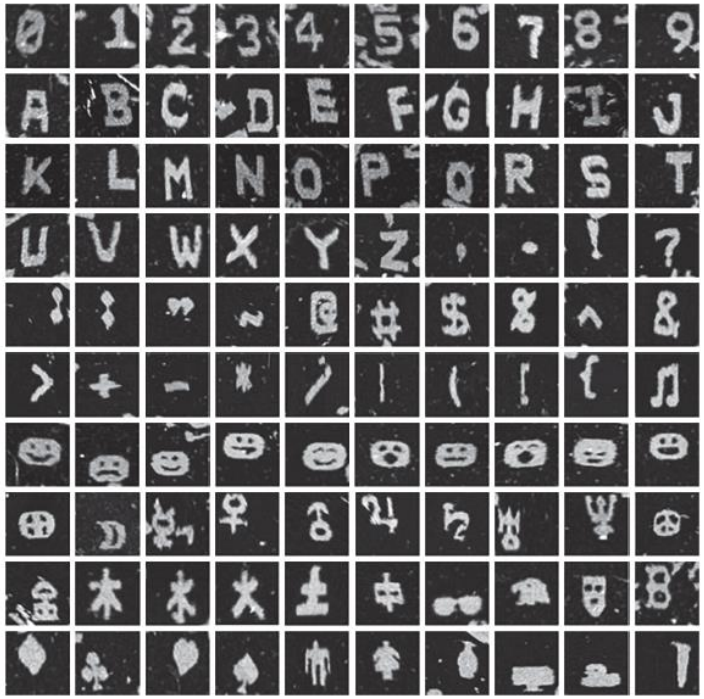


A number of approaches to produce 3D structures:

- Association of identical symmetrical DNA three-point-star or five-point-star tiles
- Rolling of DNA origami sheets through crossover junctions

Ke Y., *Science*, 338, 1167

# Origami and Brick assembly: critical points to be addressed



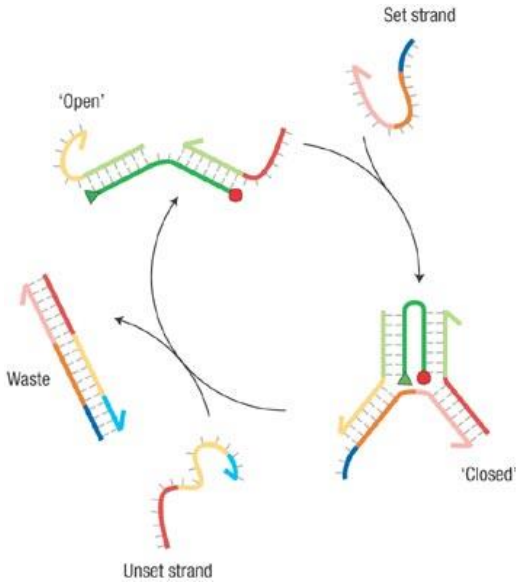
- Their need for hundreds of DNA strands
- The synthesis cost is rapidly decreasing but it is still a issue.
- **Scalability,** limitation to application that requires few micrograms of material

Li, W. Et al. J. Am. Chem. Soc. 136, 3724–3727 (2014).

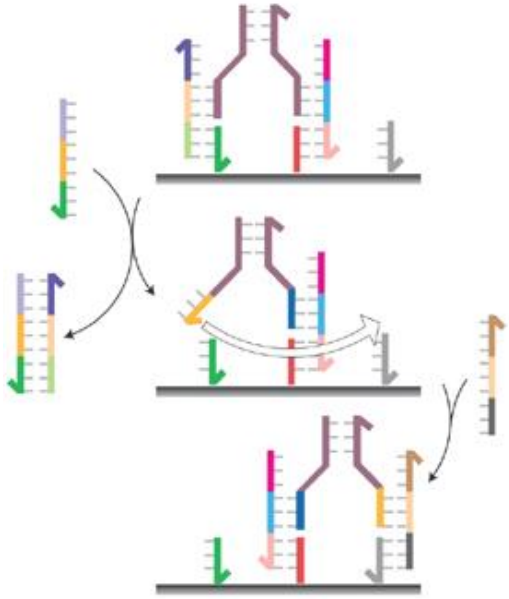
# Functional DNA Nanotechnology: DNA Nanomachines

DNA nanomachines are nanorobots made entirely or partially of DNA. DNA nanomachines can switch between defined molecular conformations and can be used as sensing, computing, actuating or therapeutic nanodevices.

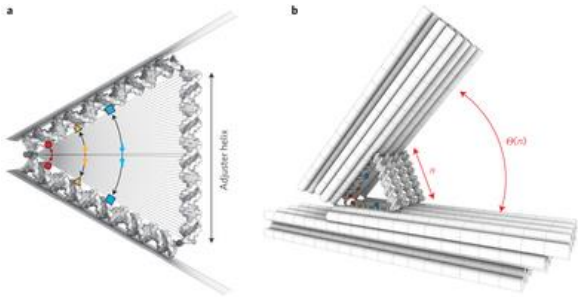
## DNA Tweezers




## DNA Walkers

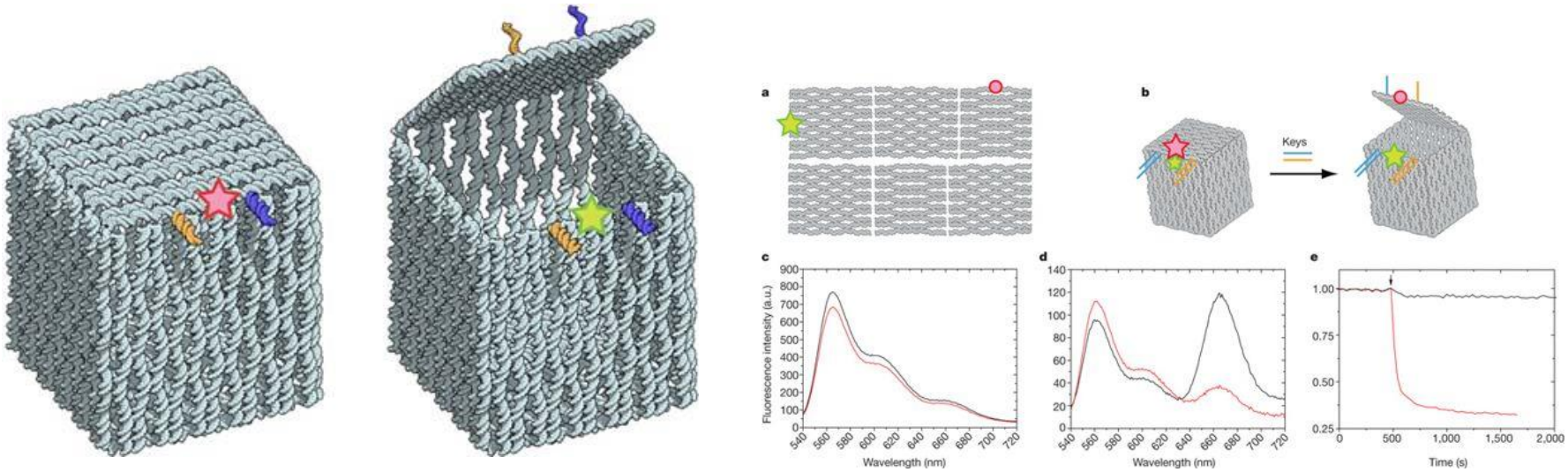


## DNA Switches



B. Yurke, A.J. Turberfield, A.P. Mills, F. C. Simmel, J.L. Neumann, Nature, 2000.
J. Bath, A.J. Turberfield, Nat. Nanotechnol., 2007.
T. Liedl, T.L. Sobey, F. Simmel, Nano Today, 2007.
Funke J. J., Dietz H. Nature Nanotechnology, 2016.

# Structural + Functional DNA Nanotechnology

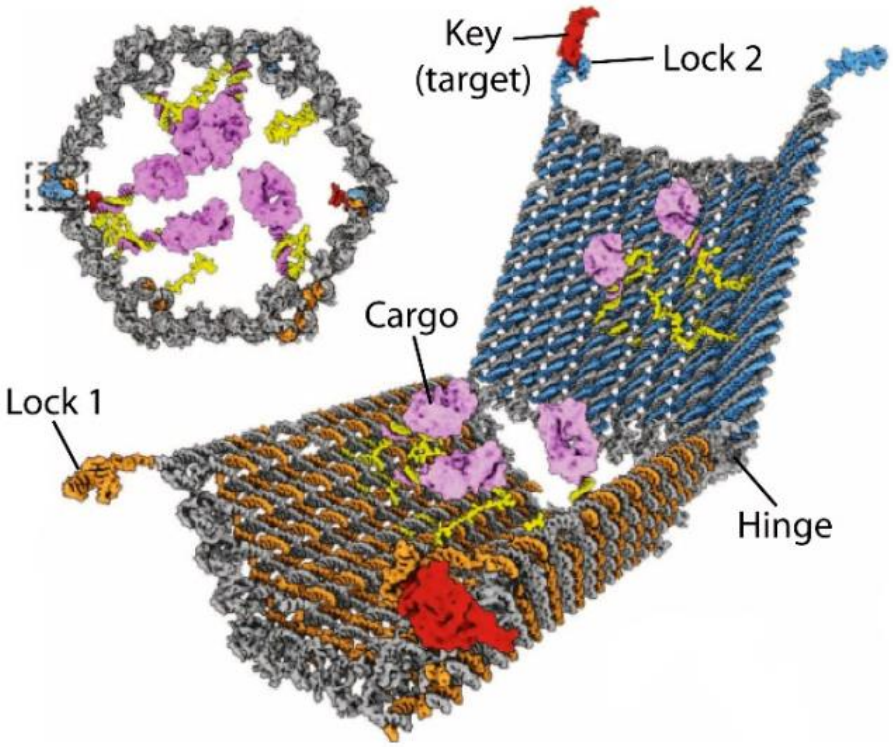



**DNA box with a controllable lid that can be opened in the presence of externally supplied DNA ‘keys’.**

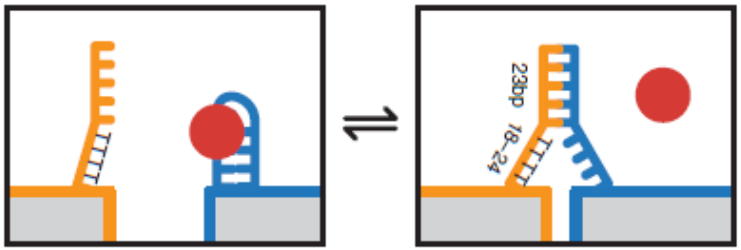
**The lids of the DNA box have the potential to be uniquely programmed to respond to complex combinations of oligonucleotide sequences**

Andersen, E.S. 2009 *Nature* 459 (7243): 73–76.

# DNA Nanorobot for Targeted Transport of Molecular Payloads

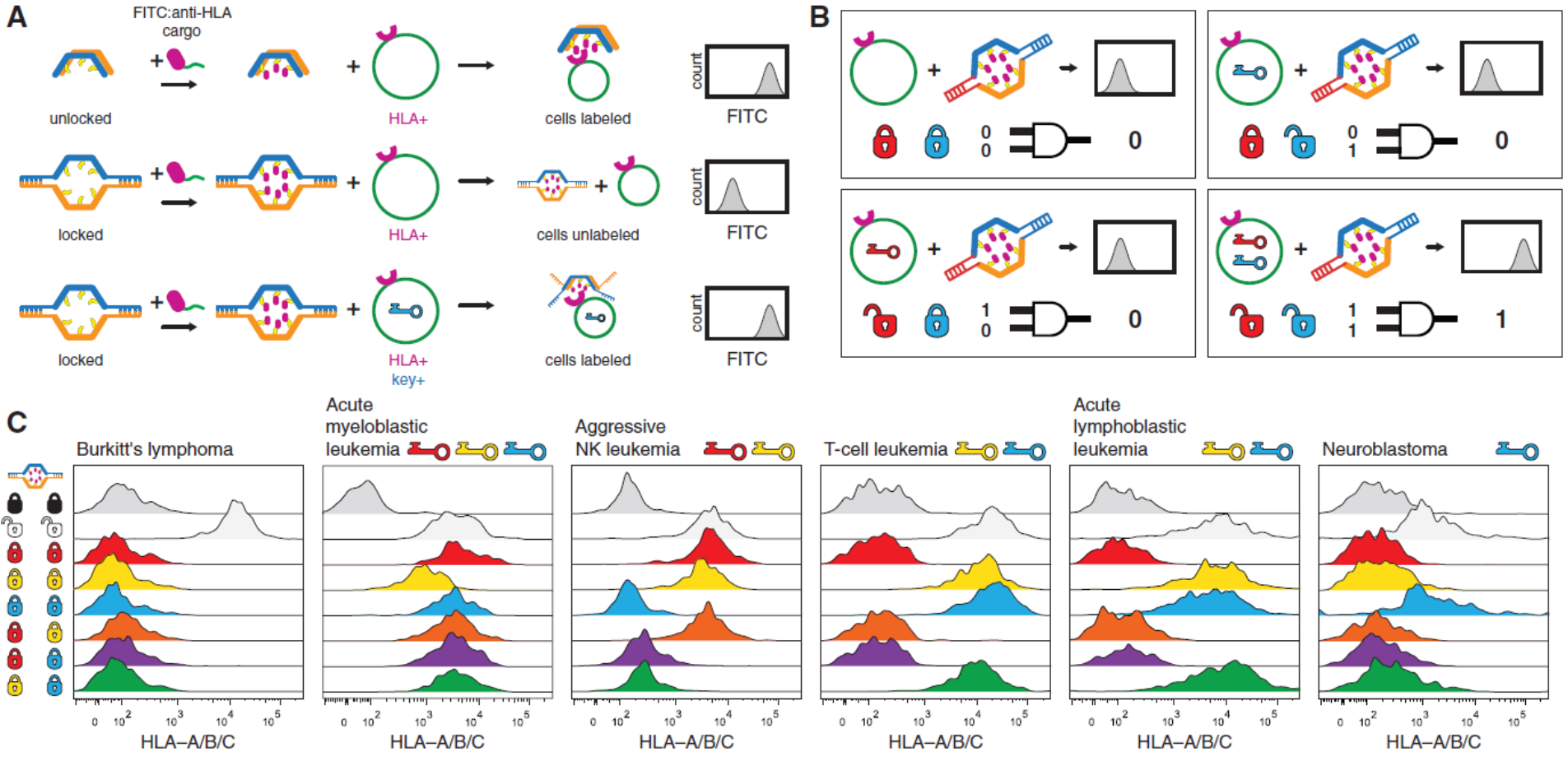



- Transport of molecular payload
- Sensing cell surface inputs and trigger activation
- Reconfiguration of its structure for payload delivery

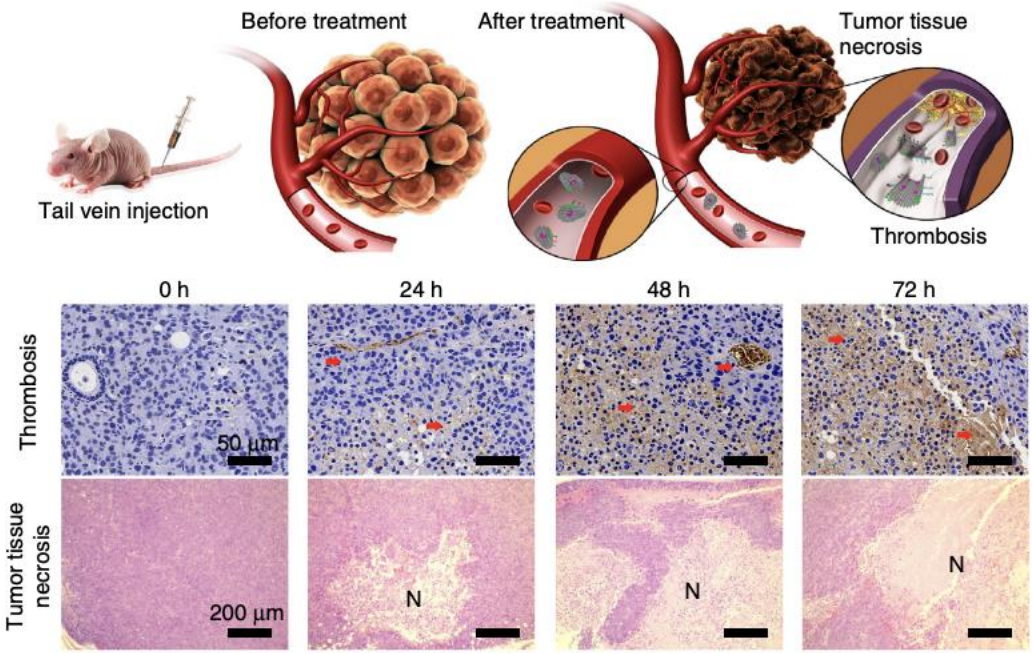



Douglas, Shawn M.; Bachelet, Ido; Church, George M. 2012 *Science* 335 (6070): 831–834.

Robots loaded with fluorescently labeled antibody fragments against human leukocyte antigen (HLA)–A/B/C were mixed with different cell types expressing human HLA-A/B/C and various “key” combinations. In the absence of the correct combination of keys, the robot remained inactive.



# DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger in vivo

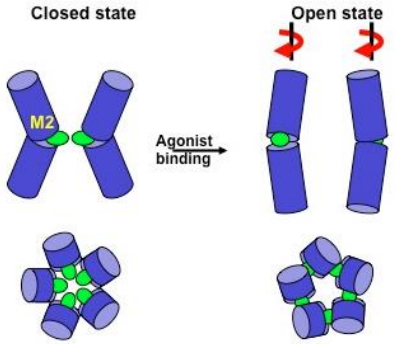


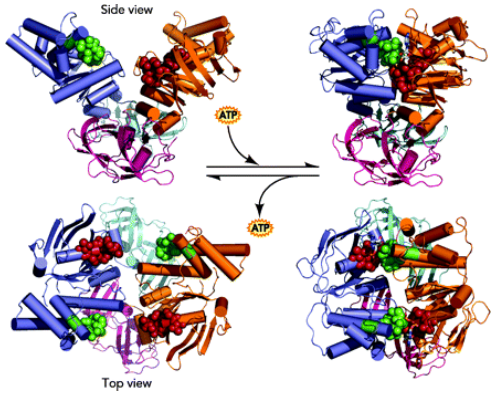

Autonomous DNA robot programmed to transport payloads and present them specifically in tumors.
Nucleolin-aptamers
Thrombin delivery to tumor site induce necrosis

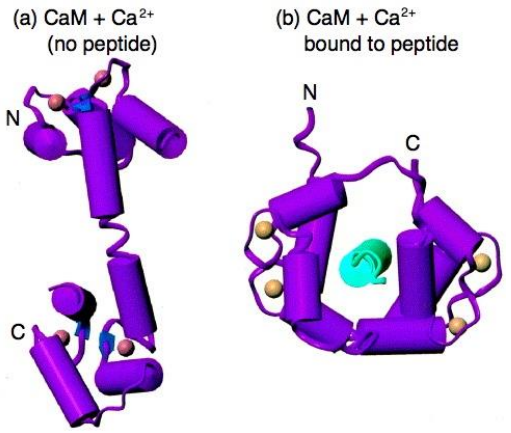
Li S., 2018 ***Nat. Biothecnol.*** 36 (3): 258-264.

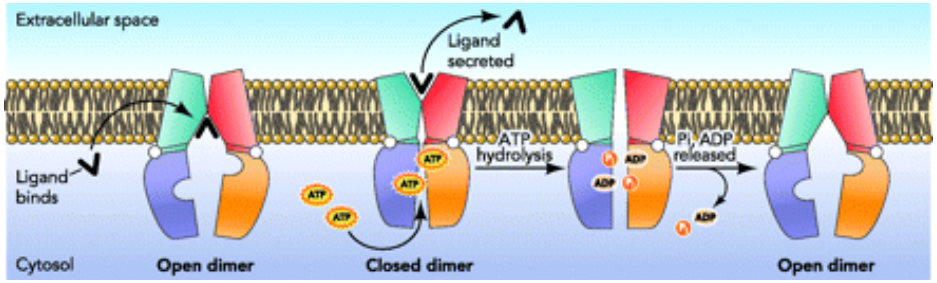
# Biomolecular switches

**Biomolecular switches made from proteins or RNA use binding-induced conformational changes as signal and functional mechanisms.**



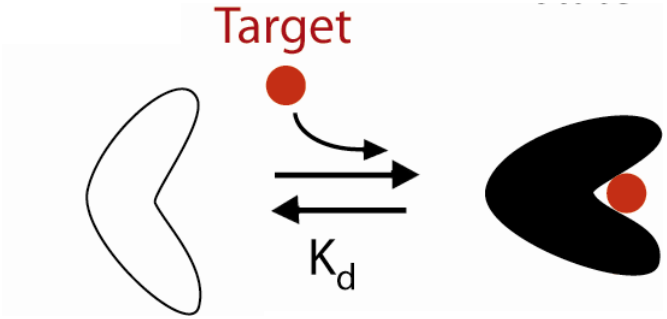



# CONCEPT: mimic nature for developing novel diagnostic and drug-delivery tools

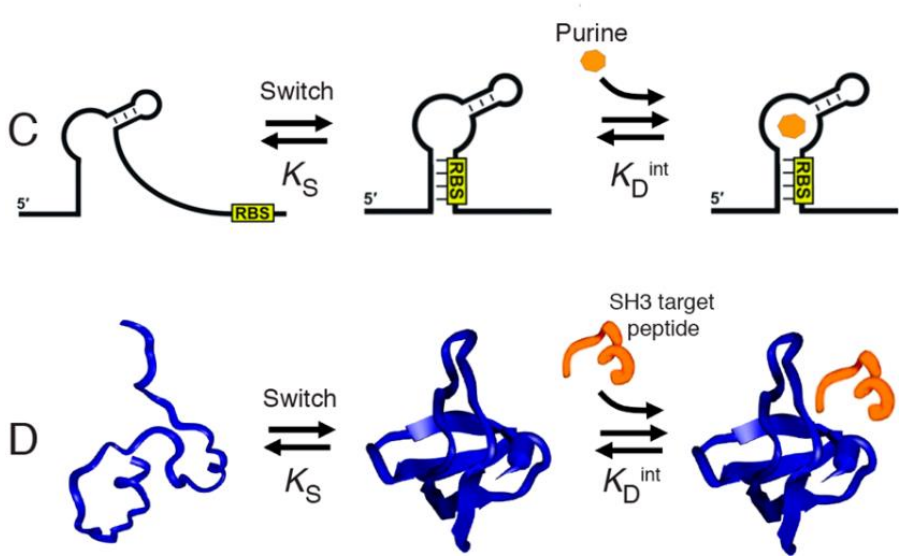
**INSPIRATION: Naturally occurring switches**




**Development of DNA based switches for biotechnology applications**

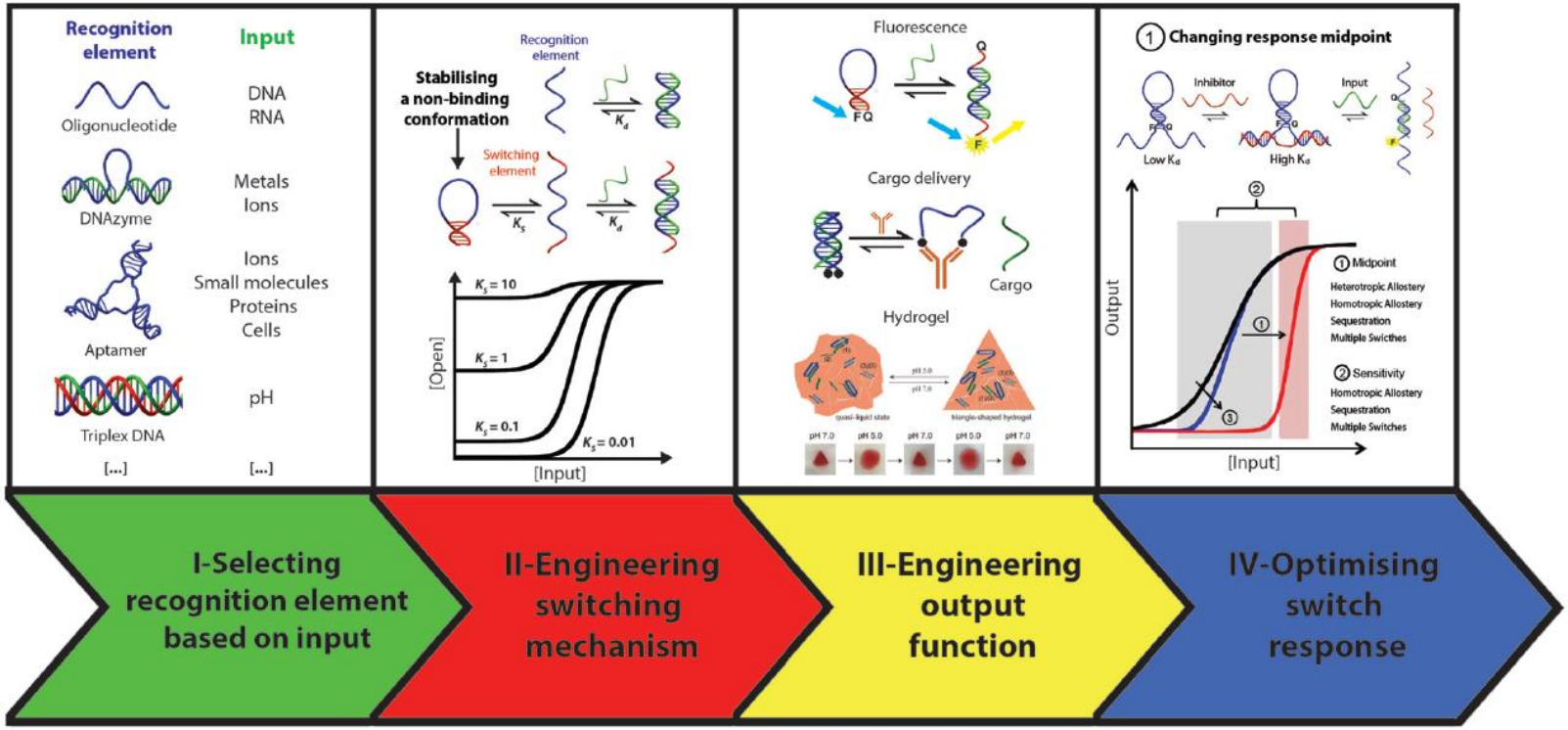
# Advantages of biomolecular switches

1- Binding-activated (not always “on”)
2- They are quantitative
3- Selective enough to work in complex biological samples
4- Switching mechanism can be engineered in any biomolecule



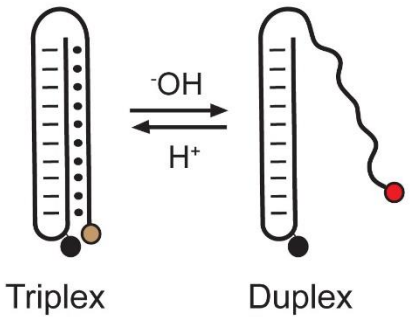

Remy, I. *et al., Science* **(1999)**
Kim, J. N. *et al. Biol. Cell.* **(2008)**
Kohn, J. *et al. Proc. Natl. Acad. Sci. U.S.A.***(2005)**

# DNA-based nanomachines design



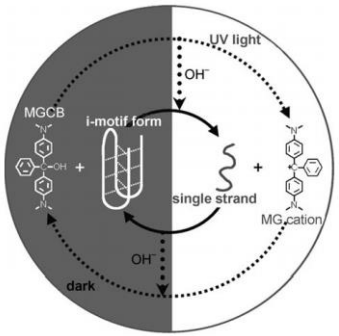
# DNA-based nanomachines: Possible inputs

## pH



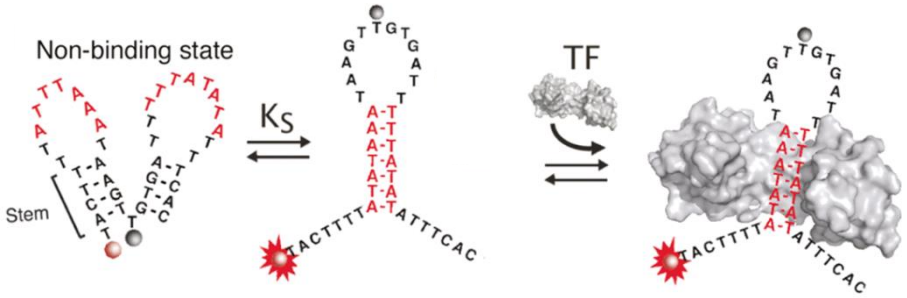

Idili, A. *et al.* JACS 2014, 136, 5836-5839

## Light



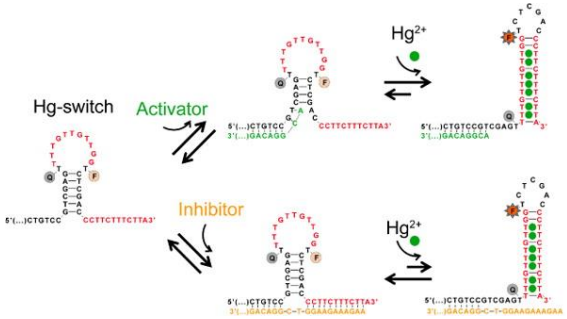

Liu, H. *et al.* Angew. Chem. Int. Ed. 2007, 46, 2515-2517

## Transcription Factor




Vallée-Bélisle, A. *et al.* J. Am. Chem. Soc. 2011, 133, 13836-9

## Small Molecule

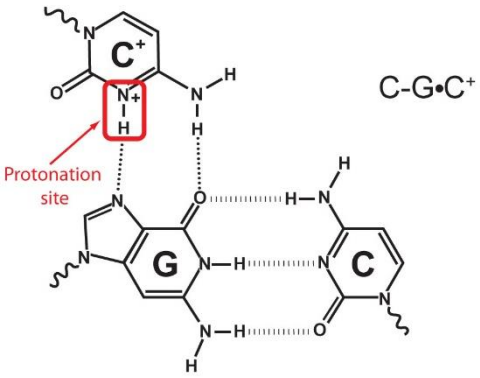



Porchetta, A. *et al.* J. Am. Chem. Soc. 2013, 135, 13238–41

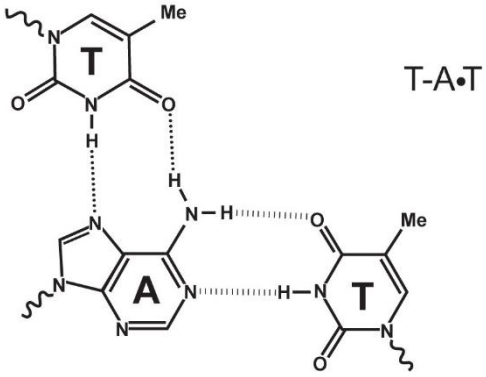
# Selecting Recognition Element based on Chemical Input

1. pH-controlled switches

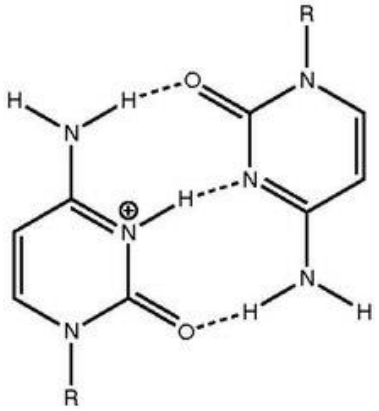
Structural information:
- *Triplex DNA (TAT, CGC triplet)*
- *i-motif (C-rich sequences)*




C-G•C+ Hoogsteen interactions are strongly pH-dependent (pKa ≈ 6)

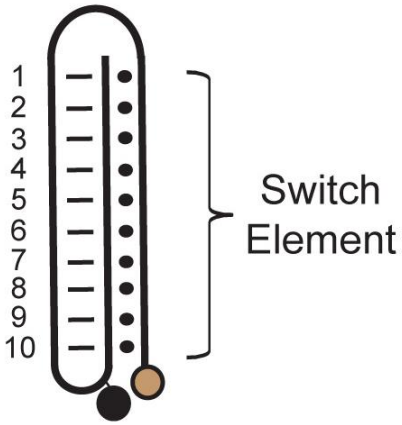


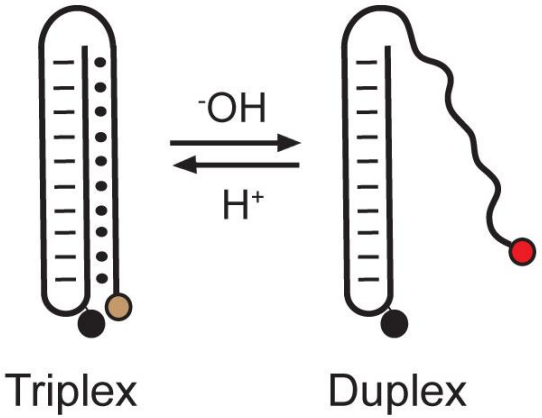

T-A•T Hoogsteen interactions are less pH-dependent (pKa ≈ 10)




C·C+ base pairing found in i-motif structures. Base-pairing energy=169.7 kJ/mol.

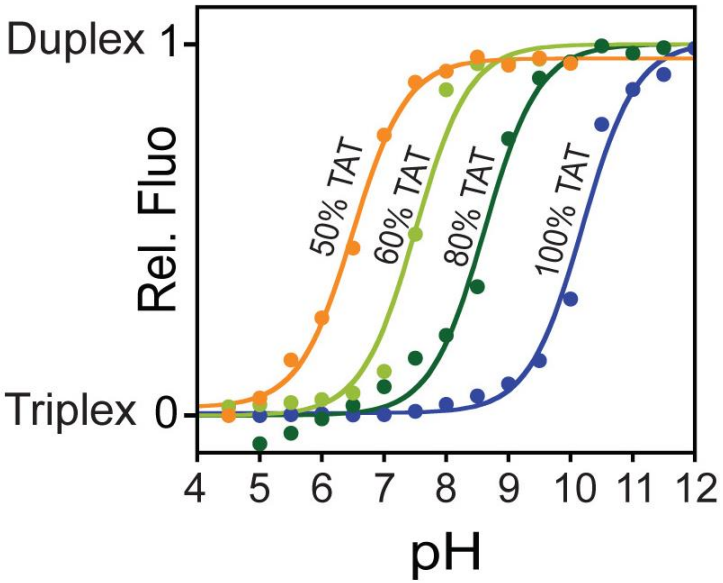
# Programmable pH-triggered DNA-based nanomachines



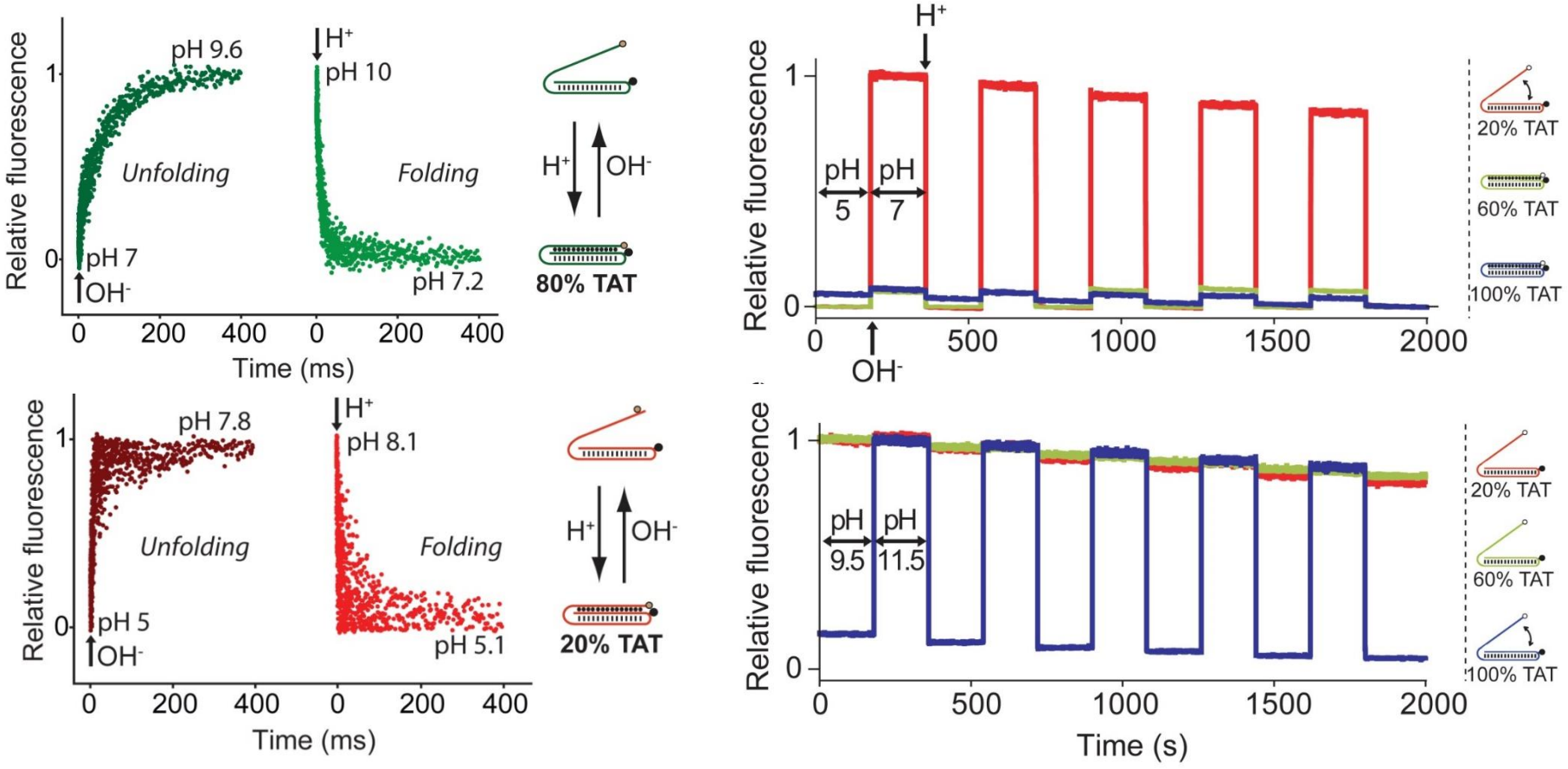

% of TAT in the Triplex

| Position | 50% | 60% | 80% | 100% |
|---|---|---|---|---|
| 1 | TAT | TAT | TAT | TAT |
| 2 | TAT | TAT | TAT | TAT |
| 3 | TAT | CGC | CGC | TAT |
| 4 | TAT | CGC | TAT | TAT |
| 5 | TAT | TAT | TAT | TAT |
| 6 | CGC | TAT | TAT | TAT |
| 7 | CGC | CGC | TAT | TAT |
| 8 | CGC | TAT | CGC | TAT |
| 9 | CGC | TAT | TAT | TAT |
| 10 | CGC | CGC | TAT | TAT |

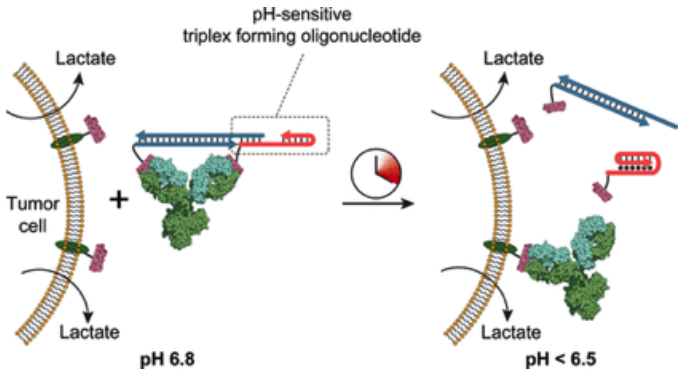



Idili A, Vallée-Bélisle A, Ricci F. **J Am Chem Soc.** 2014, 136, 5836

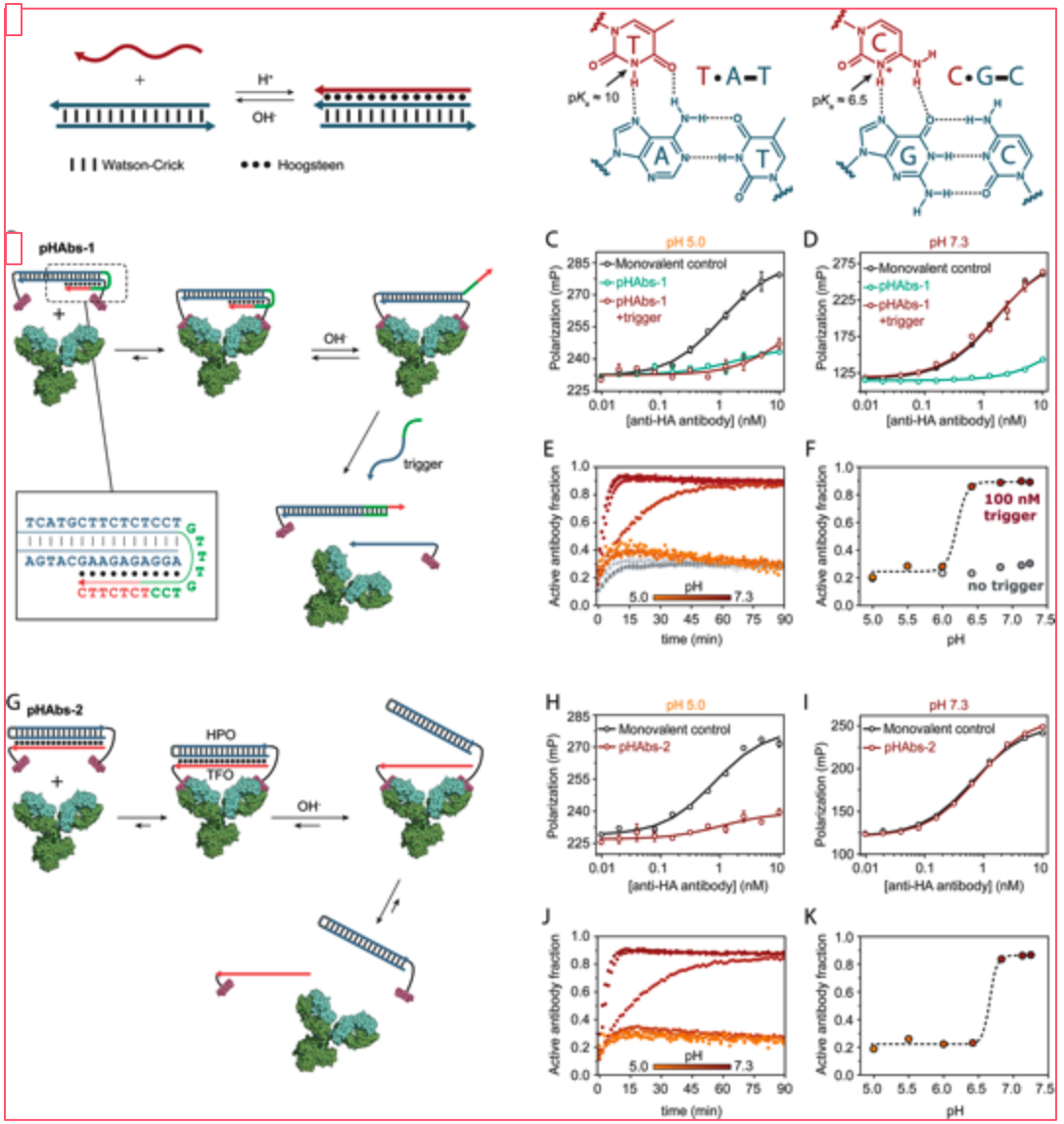
# Programmable pH-triggered DNA-based nanomachines



# pH-Based Control of Antibody Activity using programmable bivalent peptide–DNA locks

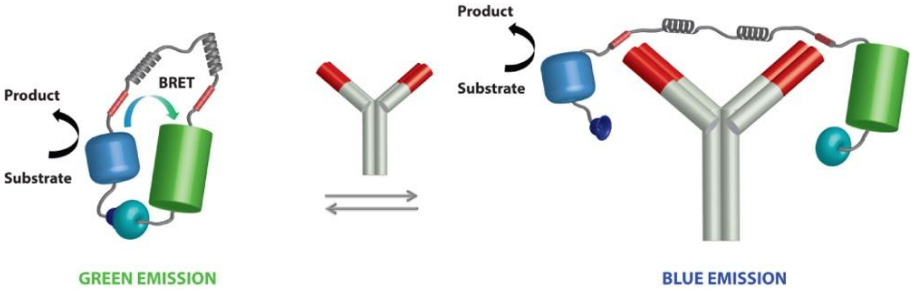



- pH-dependent DNA lock
- Detection of the specific antibody
- Targeting of tumor cells



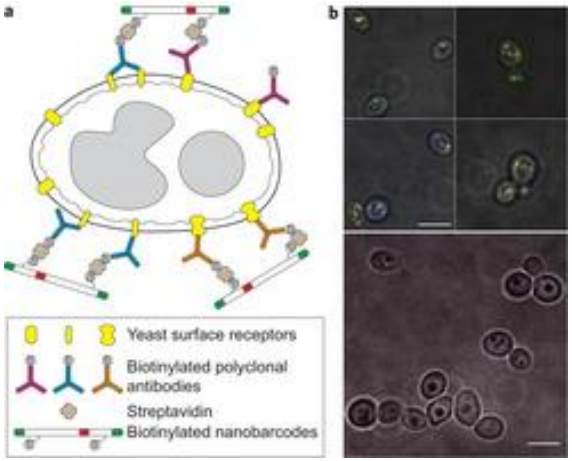

Merkx *et al.*, ***ACS Cent. Sci.*** 2020, *6*, 1, 22-31

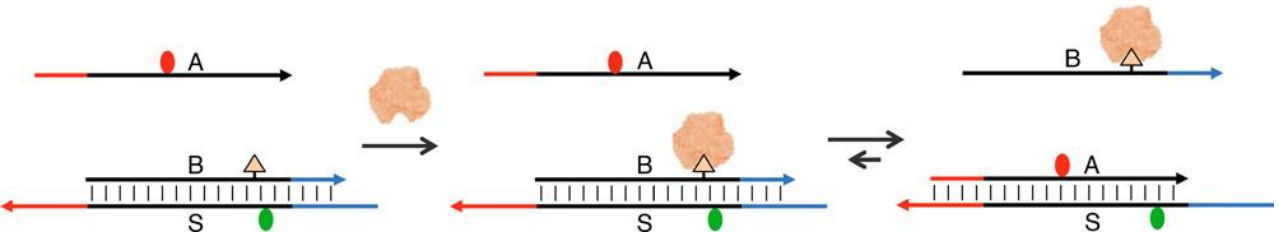
# Antibodies as triggering input for DNA-based nanomachines




Arts R. *et al.*, *Anal. Chem.*, 2016, 88, 4525–4532

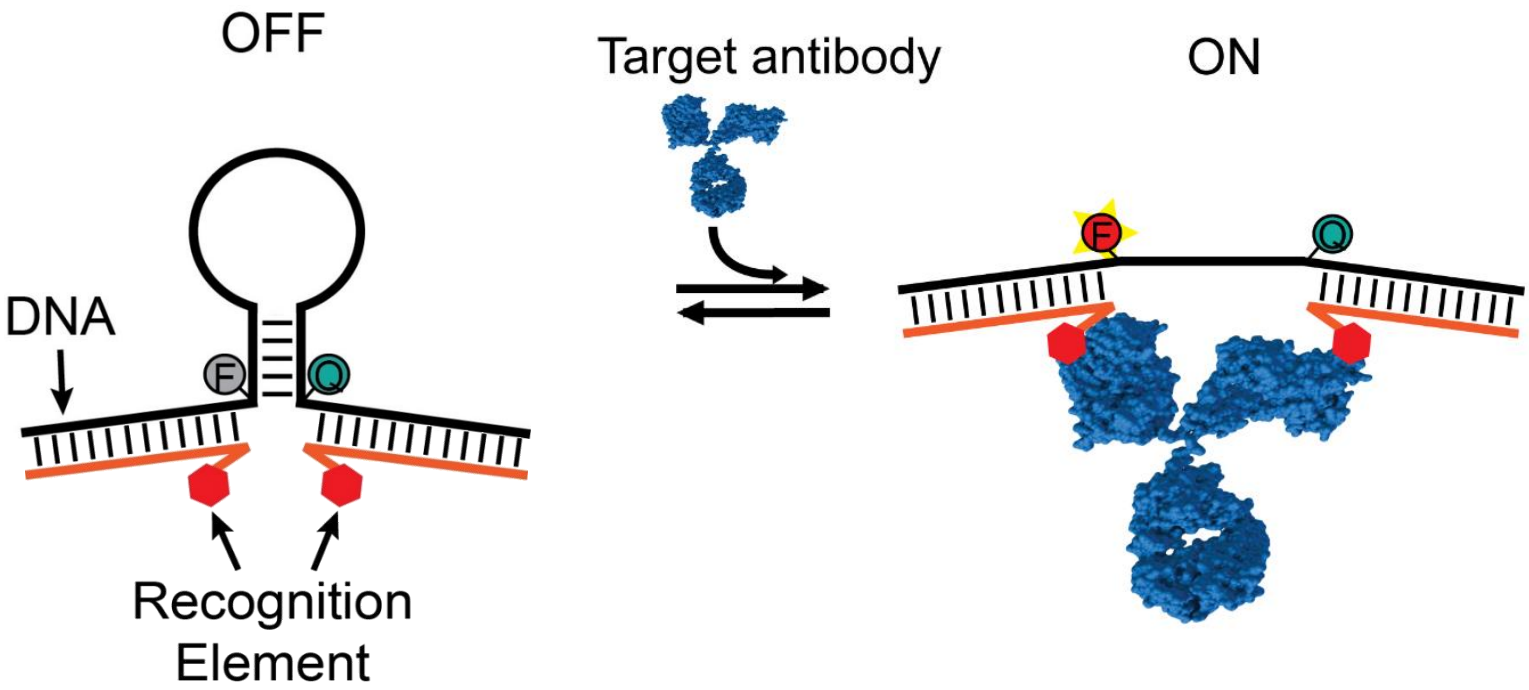
Chenxiang L. *et al.*, *Nature Chemistry, 2012, 4, 832–839*




Zhang Z. *et al.*, *J. Am. Chem. Soc.*, 2014, 136, 11115–11120

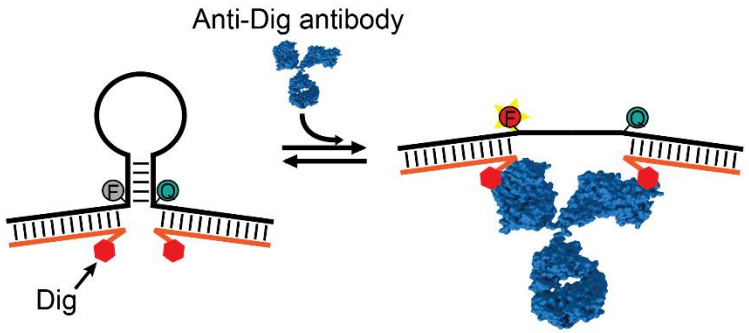
# Antibody-switch:
# A DNA-based biomolecular switch that, through a binding-induced conformational change, detects antibodies



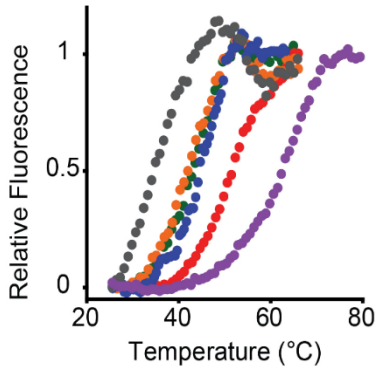

## Working principle

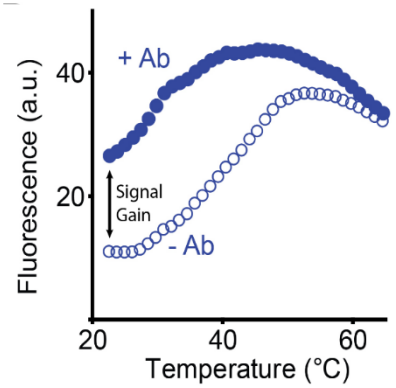
Ranallo S. *et al.*, *Angew. Chem. Int. Ed*. 54, 13214–13218 **(2015)**.

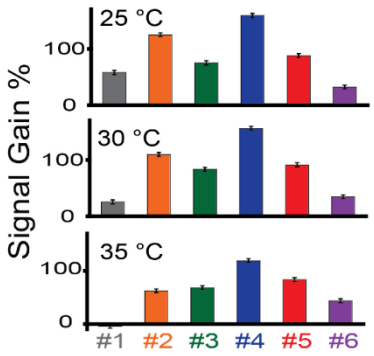
# Proof of principle of optical antibody-binding switch

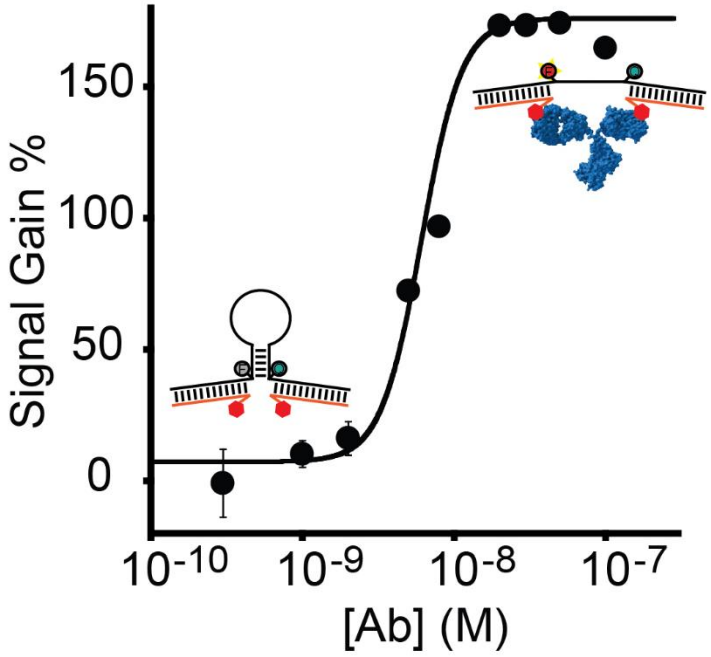



| Variant | Stem | Loop |
|---|---|---|
| #1 | 3GC+2AT | poly-T (20mer) |
| #2 | 3GC+2AT | poly-T (13mer) |
| #3 | 2GC+2AT | Random (15mer) |
| #4 | 3GC+2AT | poly-T (15mer) |
| #5 | 3GC+2AT | Random (15mer) |
| #6 | 4GC+1AT | Random (15mer) |



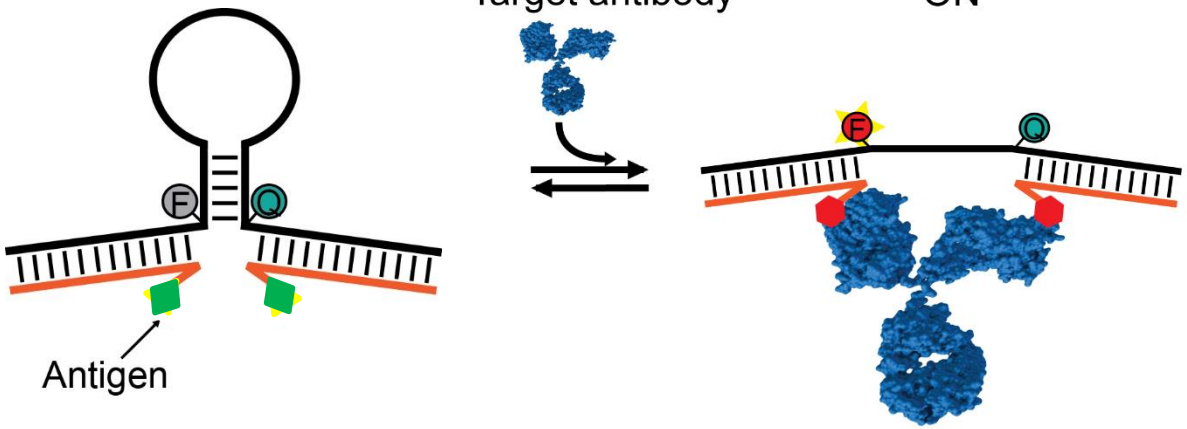



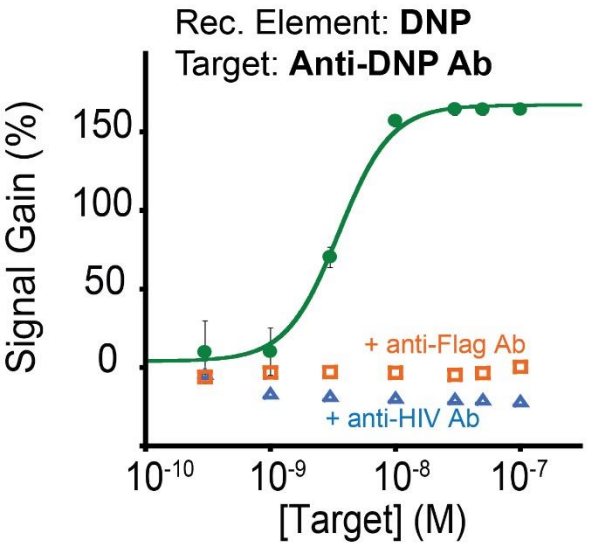

Ranallo S. *et al., Angew. Chem. Int. Ed.* 54, 13214–13218 **(2015)**.

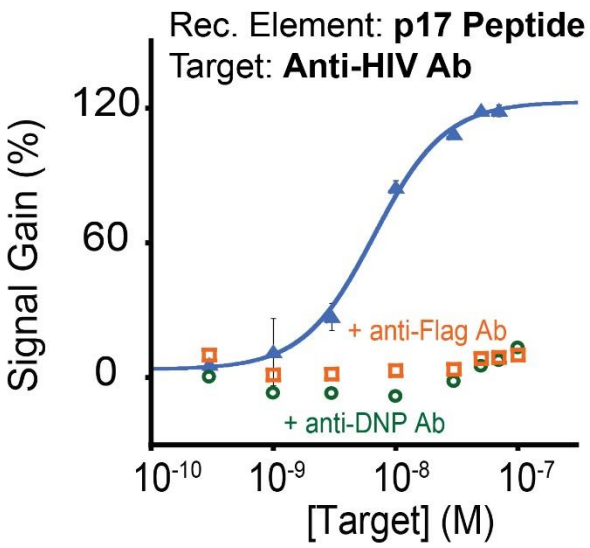
# A versatile platform

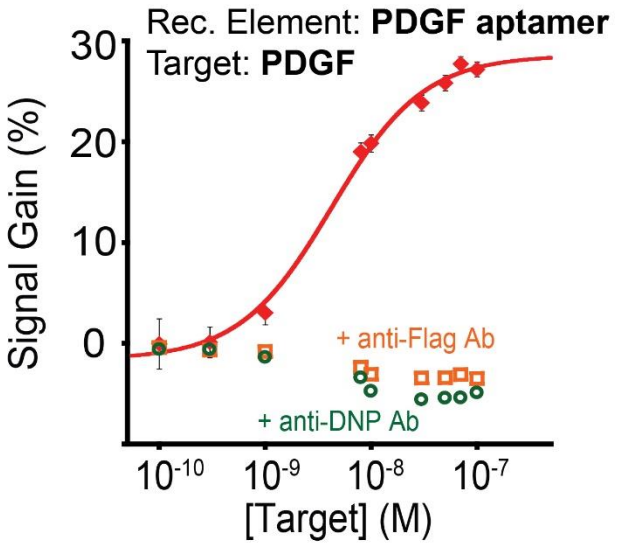
Recognition element

- = small molecule
- = peptide
- = aptamer



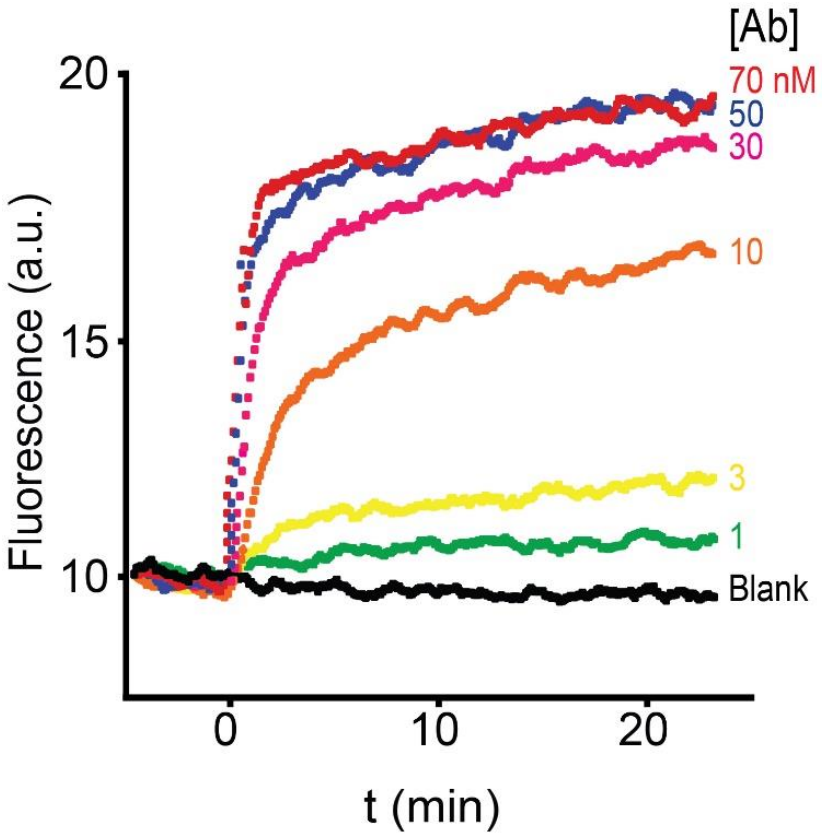



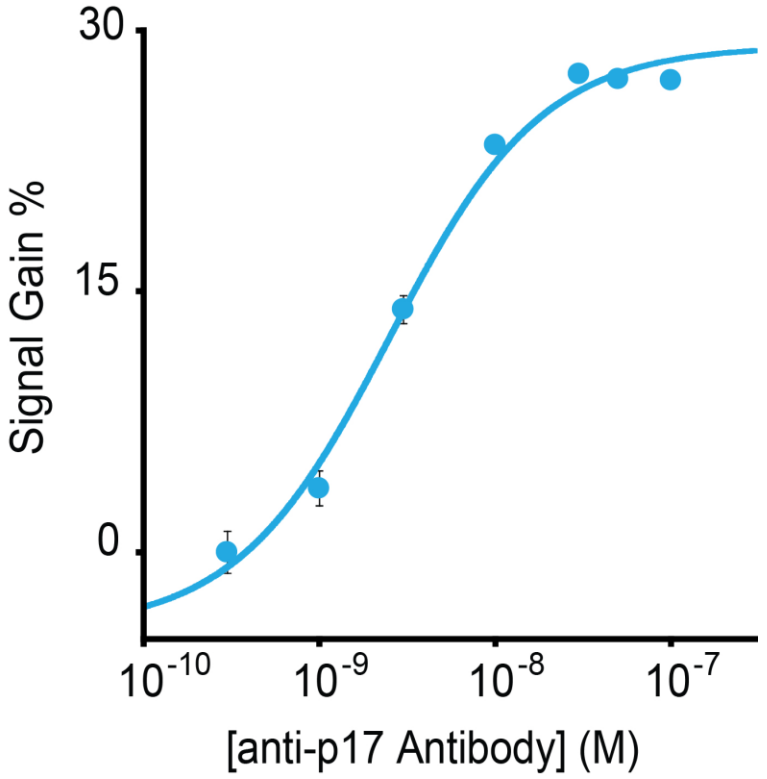




Ranallo S. *et al., Angew. Chem. Int. Ed.* 54, 13214–13218 **(2015)**.

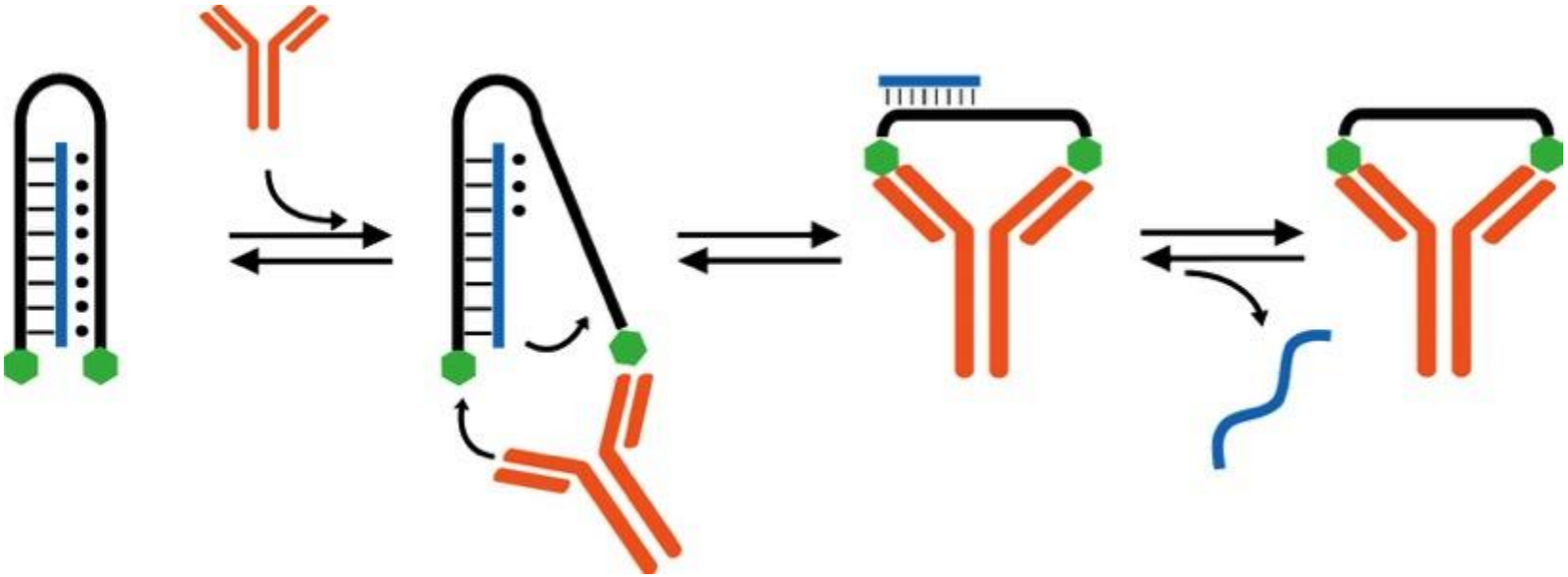
# Rapid and quantitative in complex samples (serum)



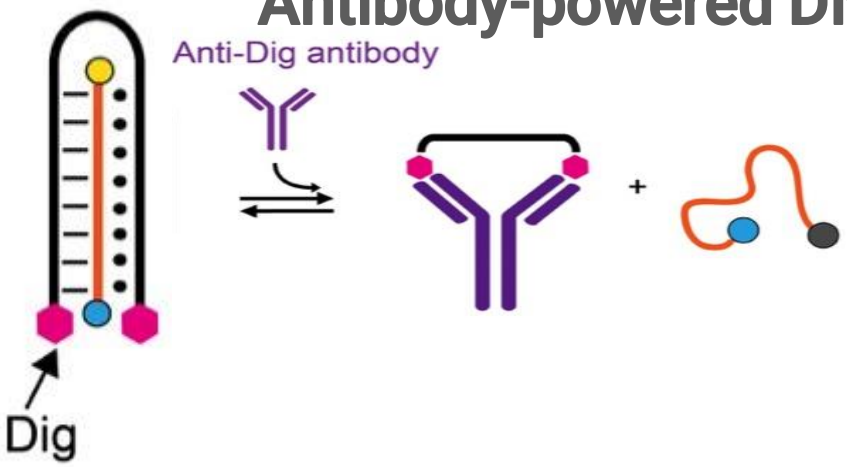

Ranallo S. *et al., Angew. Chem. Int. Ed*. 54, 13214–13218 **(2015)**.

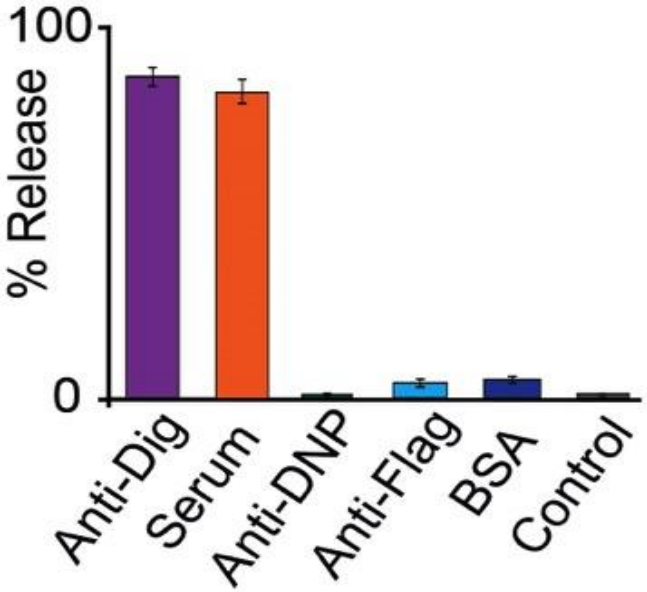
# Working principle

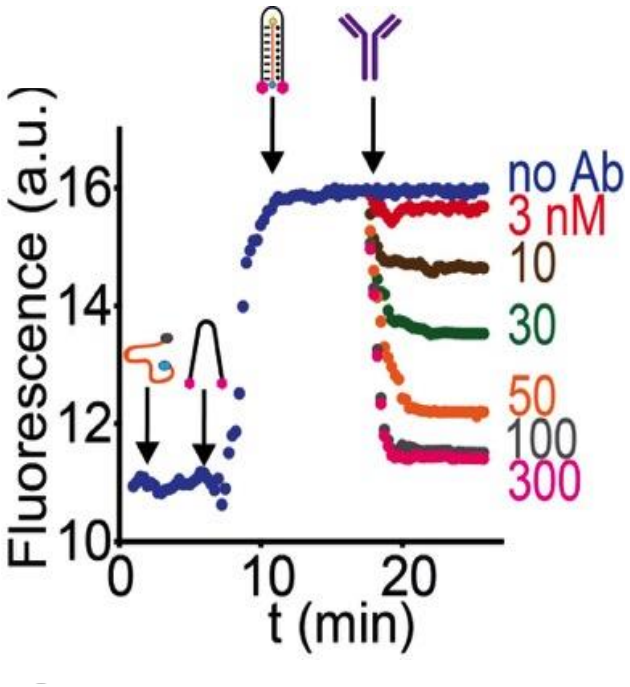


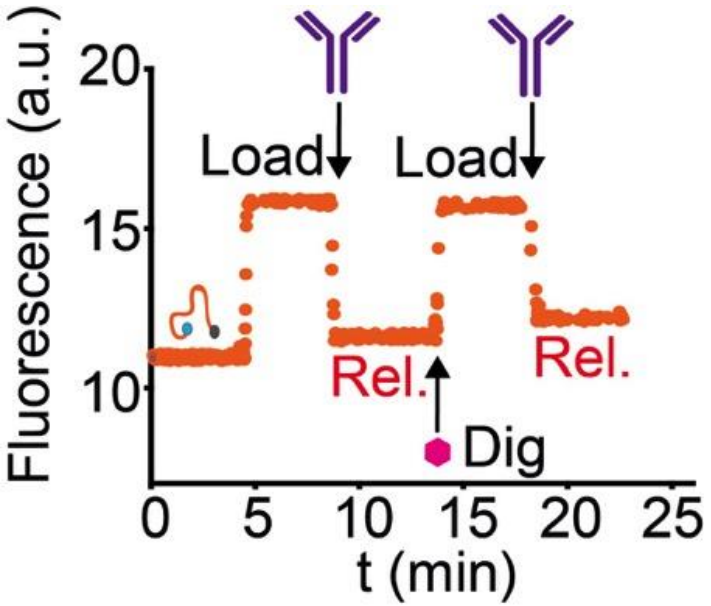
Ranallo S. *et al.*, ***Nat. Commun.*** 2017, 8, 15150.

# Antibody-powered DNA-based nanomachine



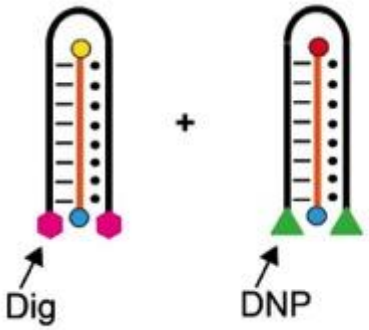



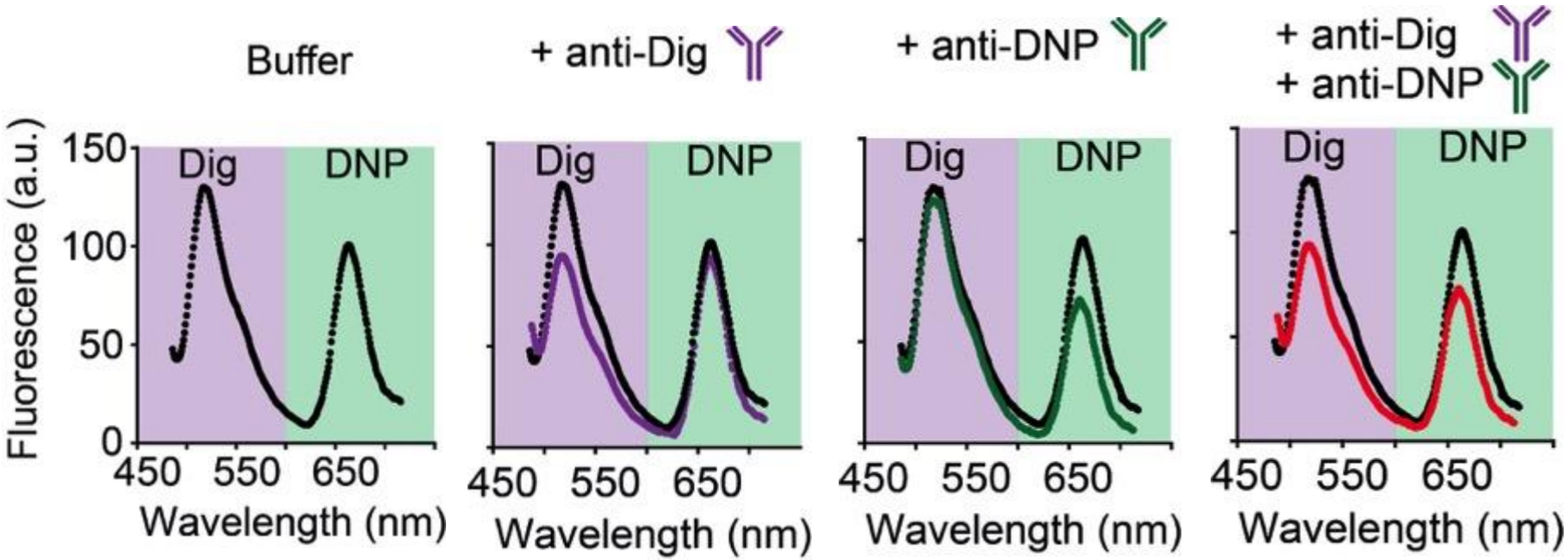




Ranallo S. *et al.*, ***Nat. Commun.*** 2017, 8, 15150.

# Antibody-powered DNA-based nanomachine

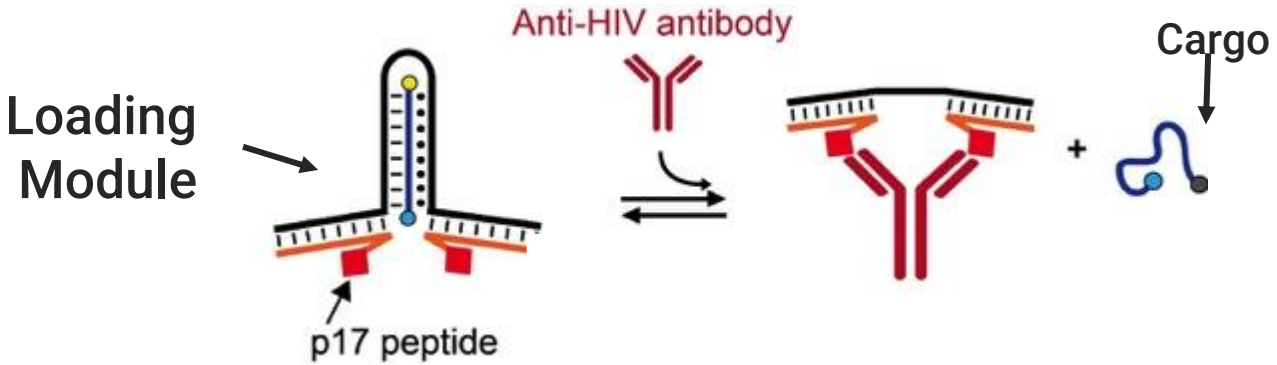


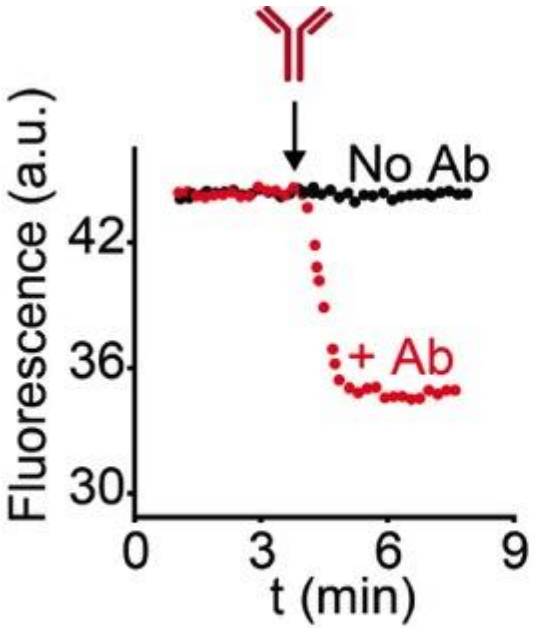

| Dye | $\lambda_{max}$ (nm) | Antigen |
|---|---|---|
| FAM | 517 | Dig |
| Quasar | 660 | DNP |

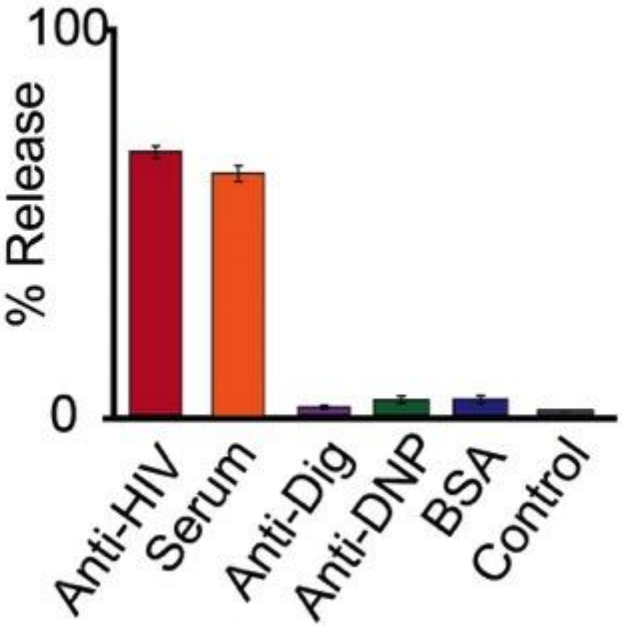


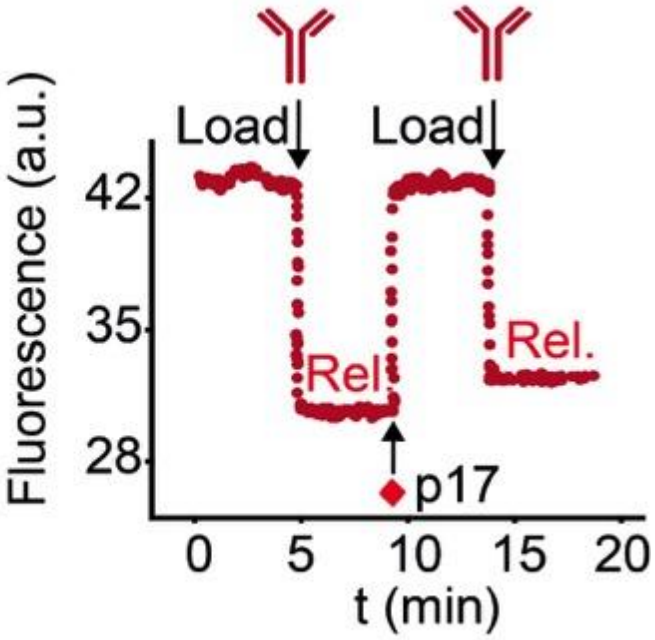

Ranallo S. *et al.*, ***Nat. Commun.*** 2017, 8, 15150.

# Modular antibody-powered DNA nanomachine



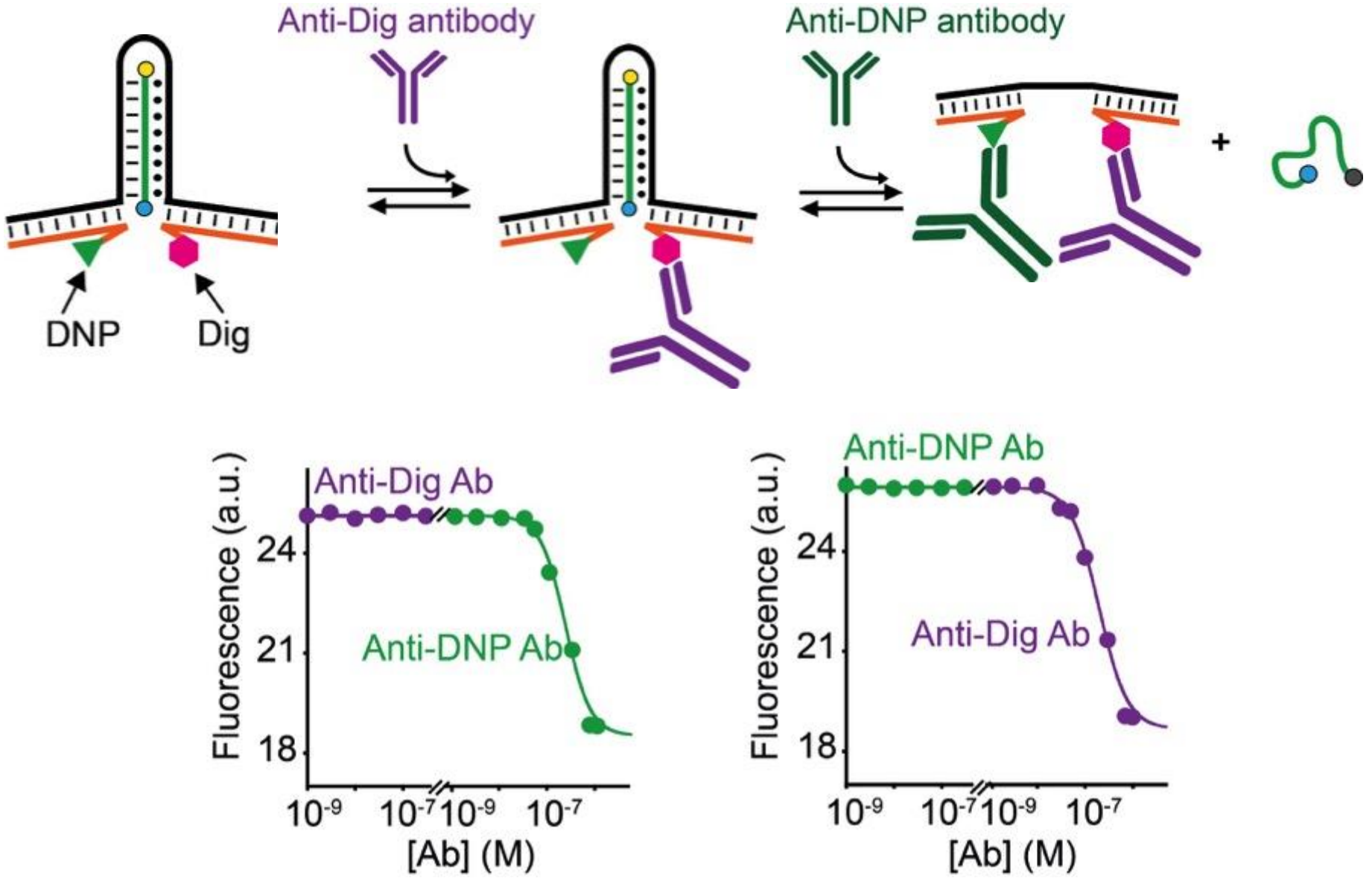




Ranallo S. *et al.*, ***Nat. Commun.*** 2017, 8, 15150.

# AND Logic Gate DNA nanomachine




Ranallo S. *et al.*, ***Nat. Commun***. 2017, 8, 15150

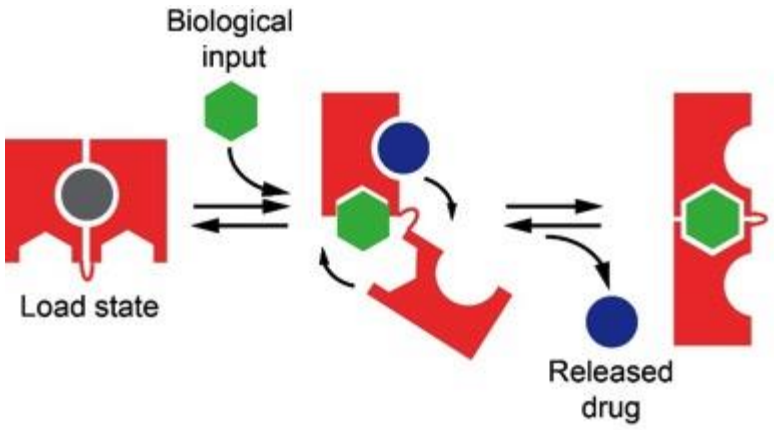
# Antibody-powered nanomachine

- ✓ Antibody-powered DNA-based nanomachines able to load and release a cargo in a controlled fashion.
- ✓ These nanomachines may prove of utility in a range of applications, including point-of-care diagnostics, controlled drug-release and in-vivo imaging.

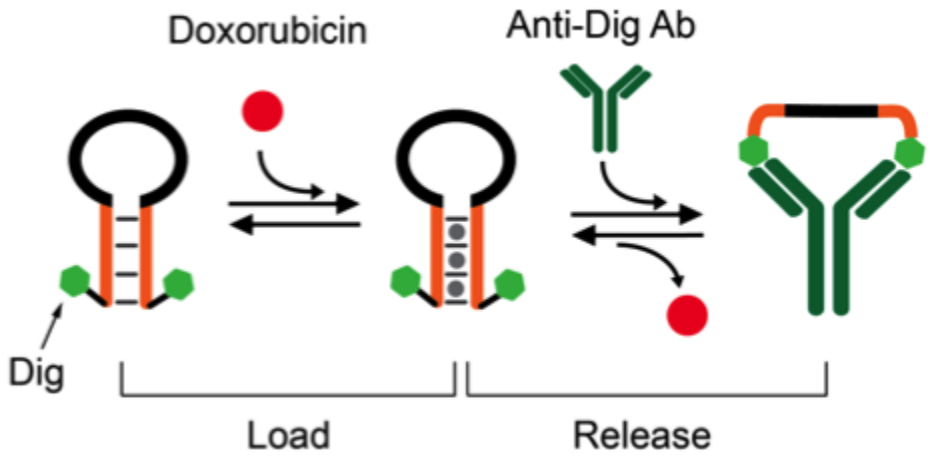
## Antibody triggers the release of Doxorubicin loaded in a DNA nanomachine


Rossetti M. *et al.*, ***Chem. Scie.*** 2017, 8, 914-920.

# Working principle

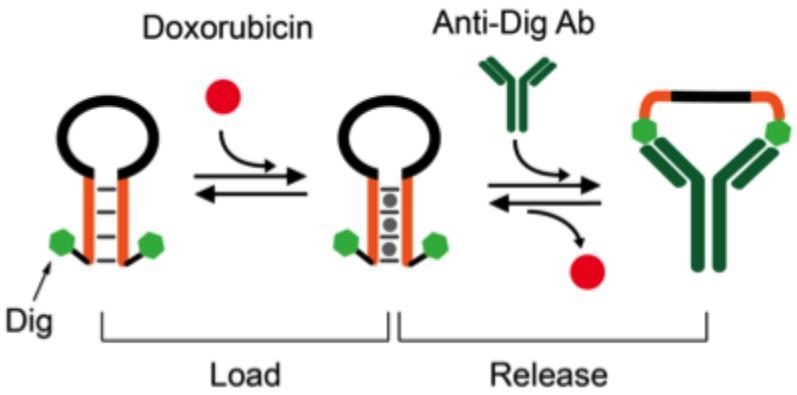


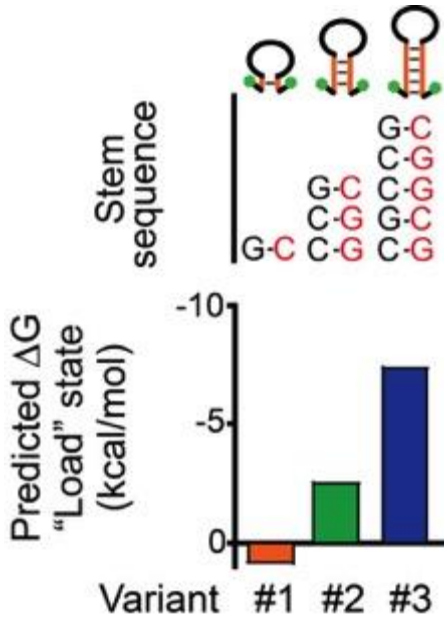

Rational design of a new class of DNA-based nanomachines *allosterically regulated* by specific biological targets, able to release a molecular cargo in a controlled fashion.

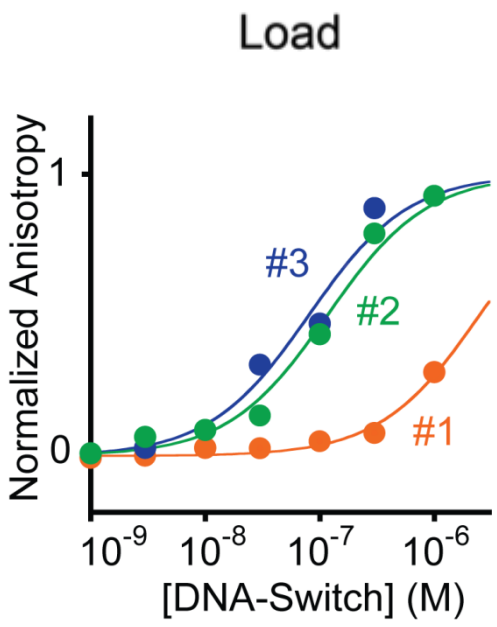


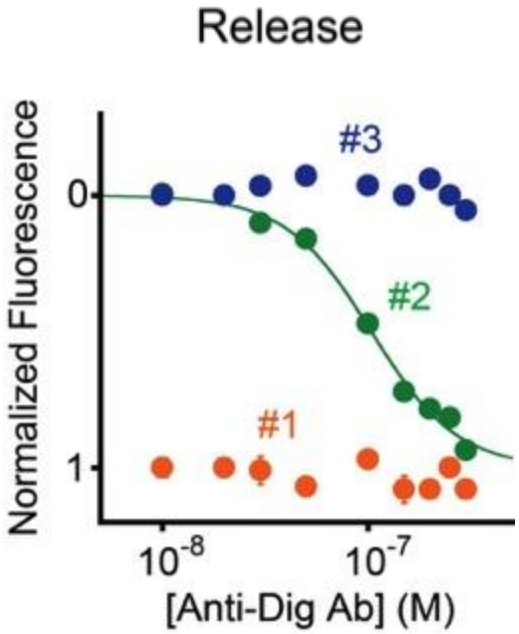

Rossetti M. *et al.*, ***Chem. Scie.*** 2017, 8, 914-920.

# DNA-cargo switches activated by Antibodies

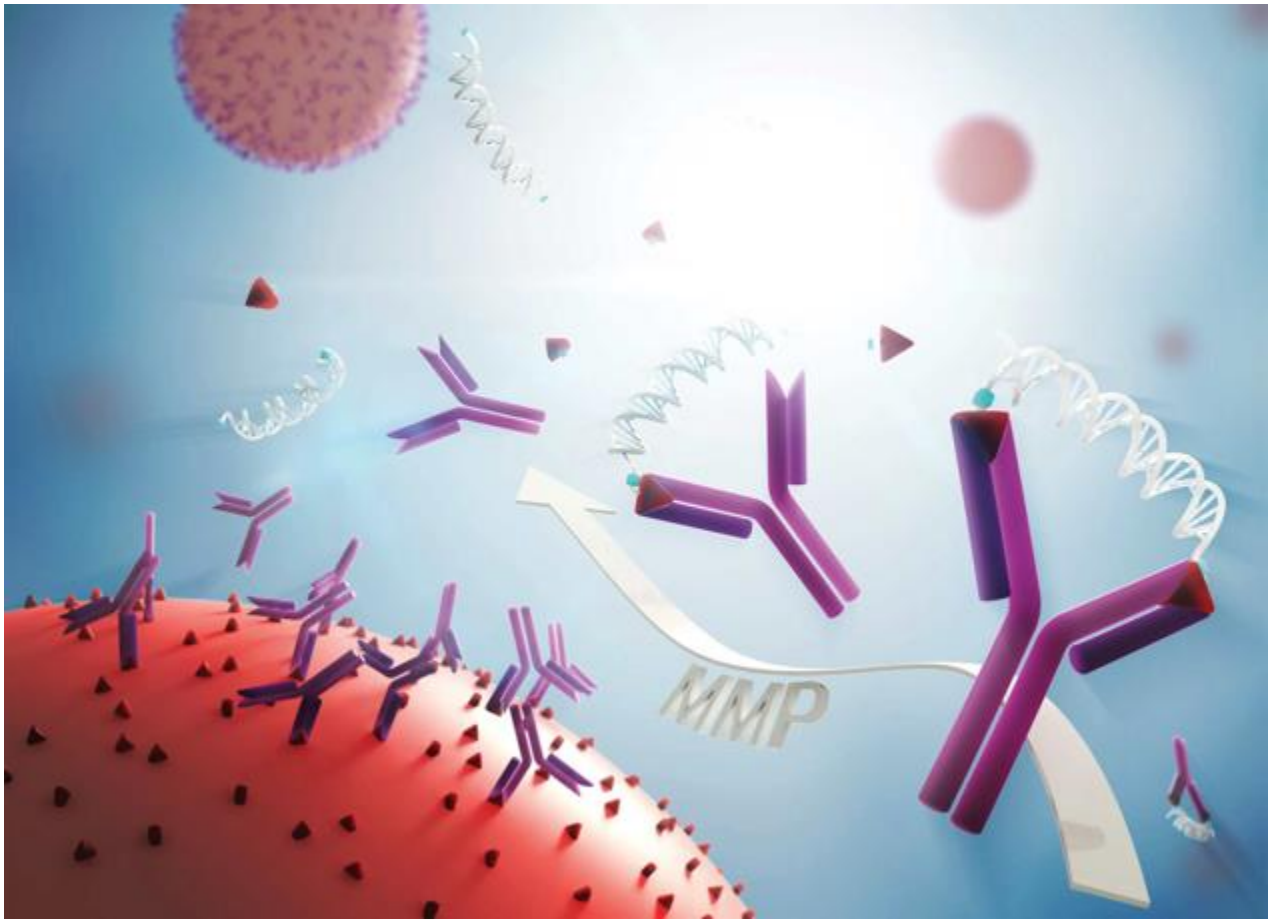


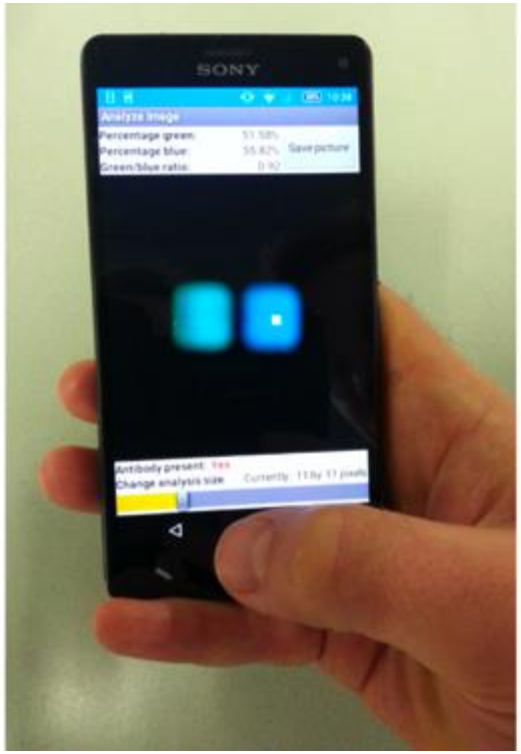

Rossetti M. *et al.*, ***Chem. Sci.*** 2017, *8*, 914-920.




# Antibody-Controlled Reactions, Logic Gates, and Circuits

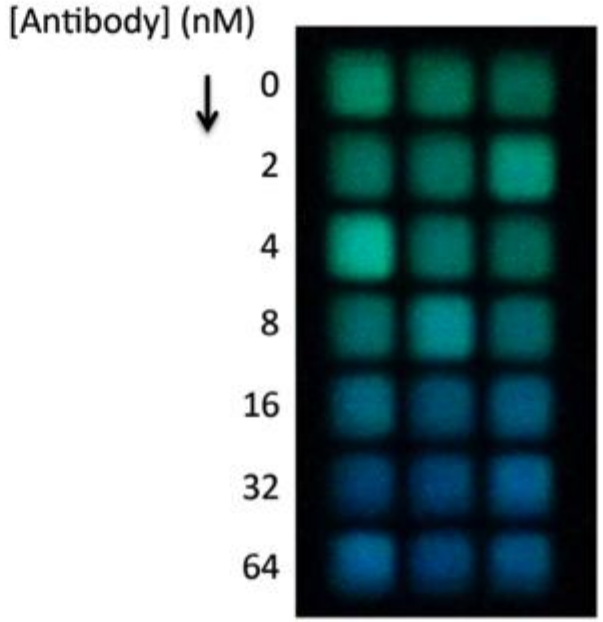
Merkx M. *et al.*, ***Org. Biomol. Chem.*** 2013, *11*, 7642–7649

# Detection of Antibodies in Blood Plasma Using Bioluminescent Sensor Proteins and a Smartphone (LUMAB sensor)




➢ Sens )

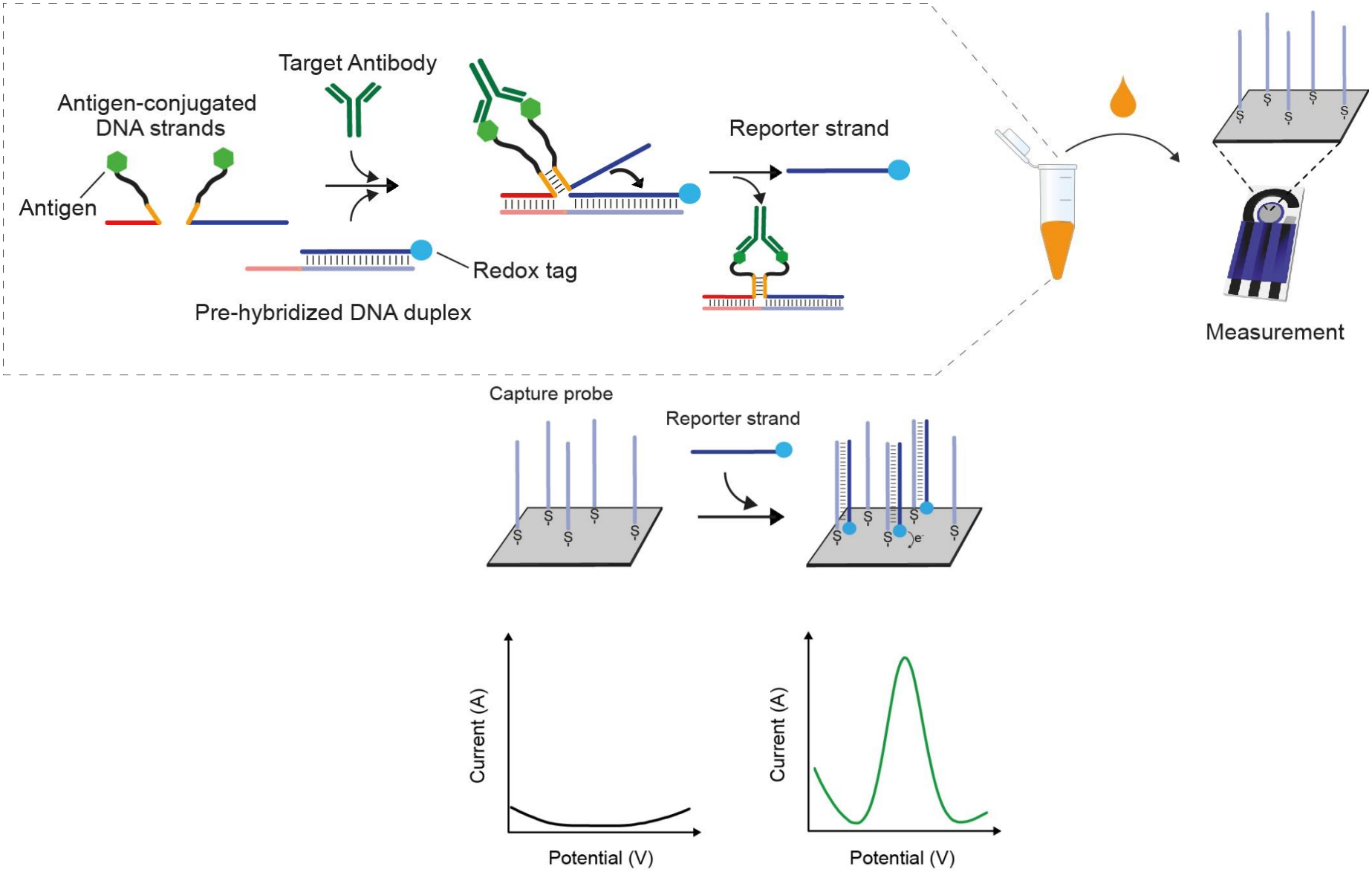
➢Bindi iteraction betwee




Change in color of the emitted light from green-blue to blue

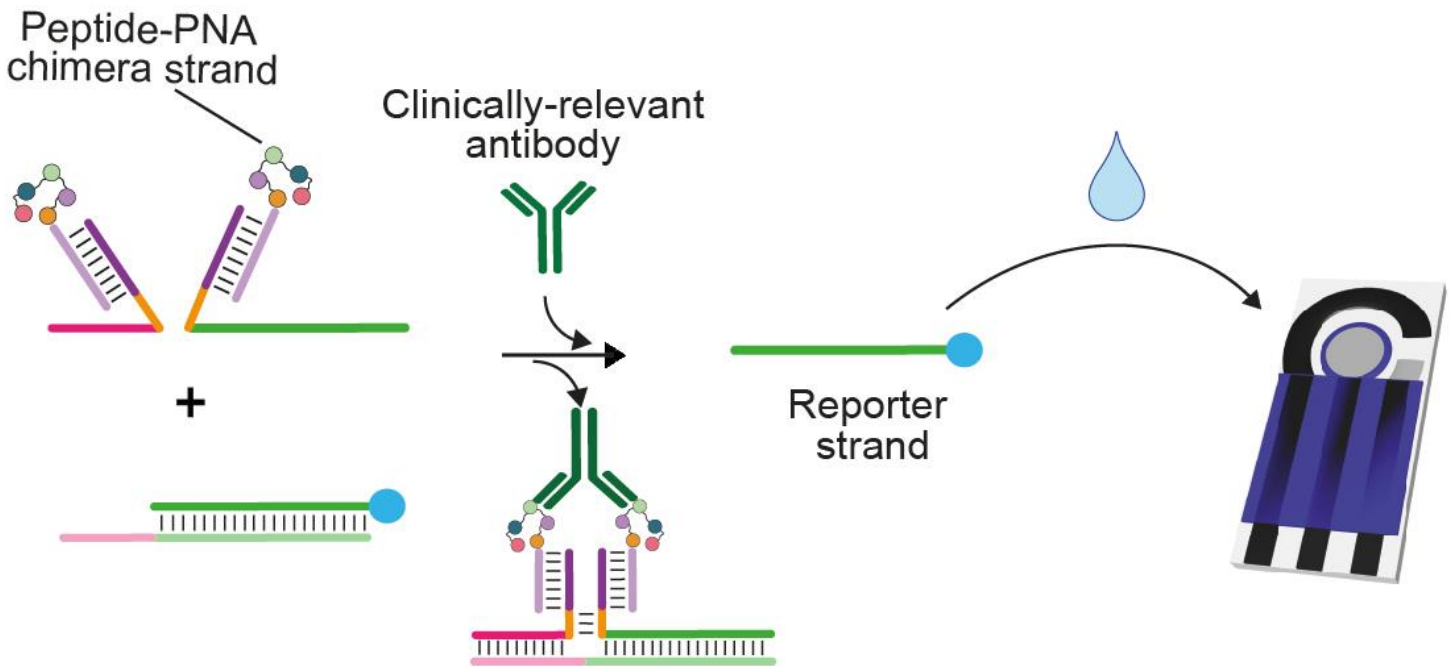
Merkx *et al.*, ***Anal. Chem.*** 2016, *88*, 4525−4532

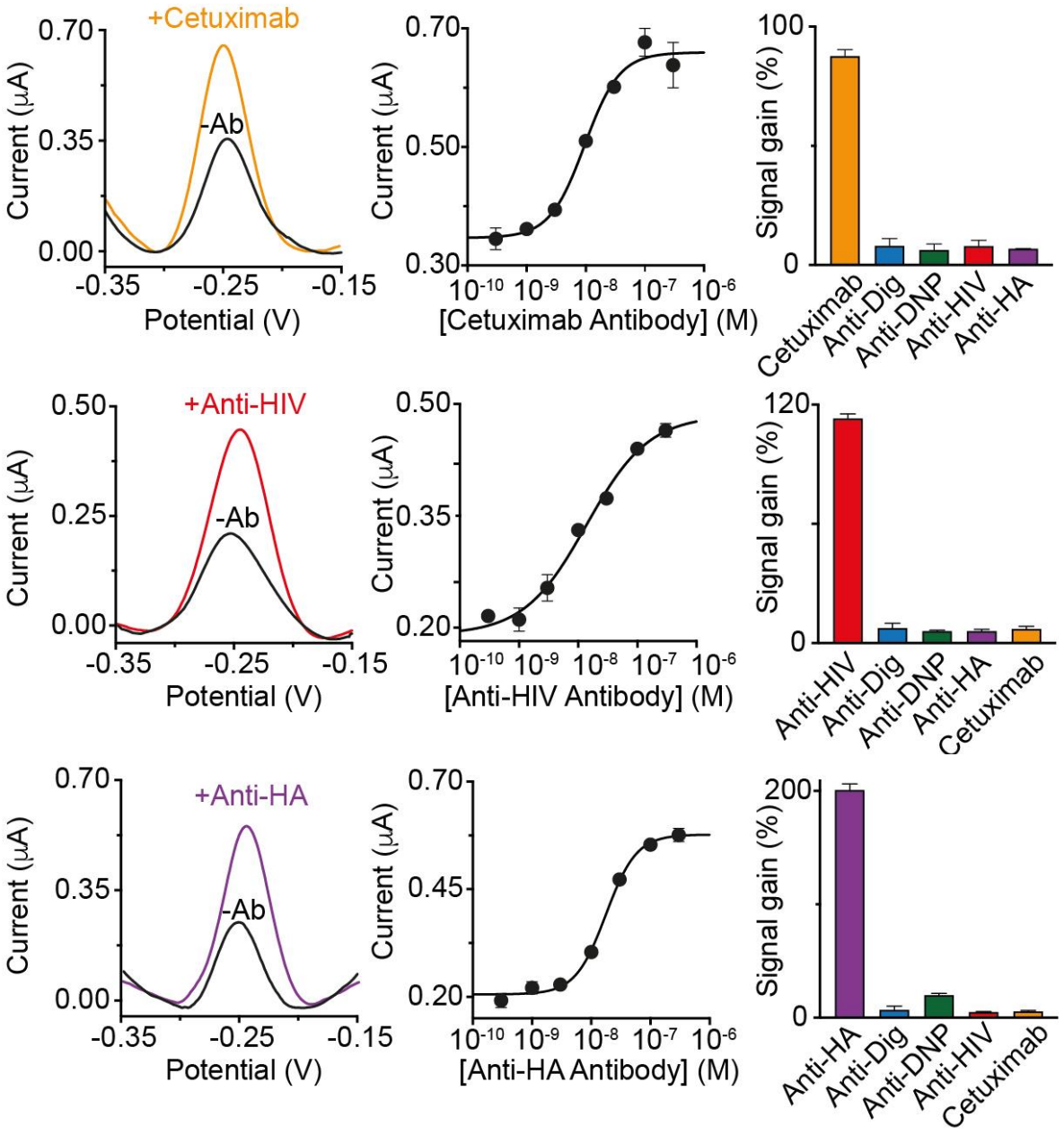
# Electrochemical DNA-based Platform for antibody detection



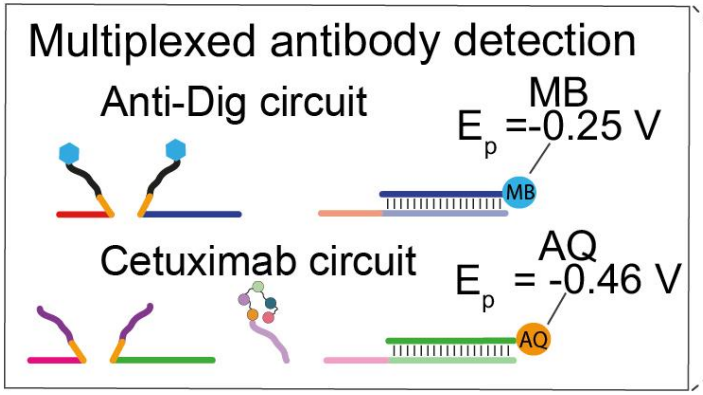

Bracaglia *S.*, Ranallo S. *et al.*, **ACS Sens.** 2021, 6, 2442−2448

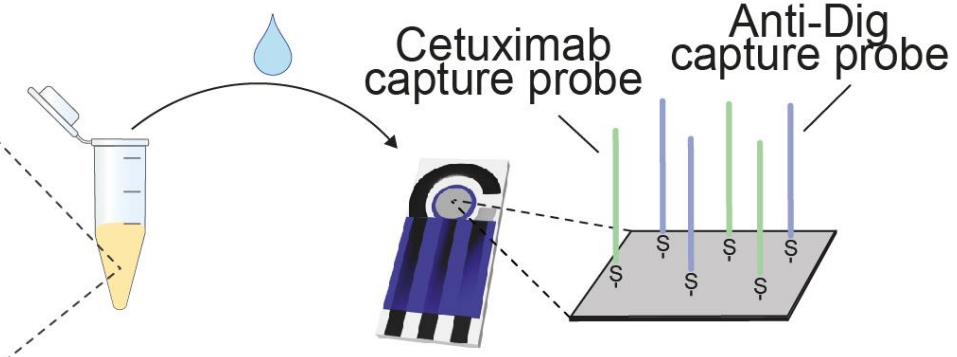
# Electrochemical platform: detection of clinically-relevant antibodies

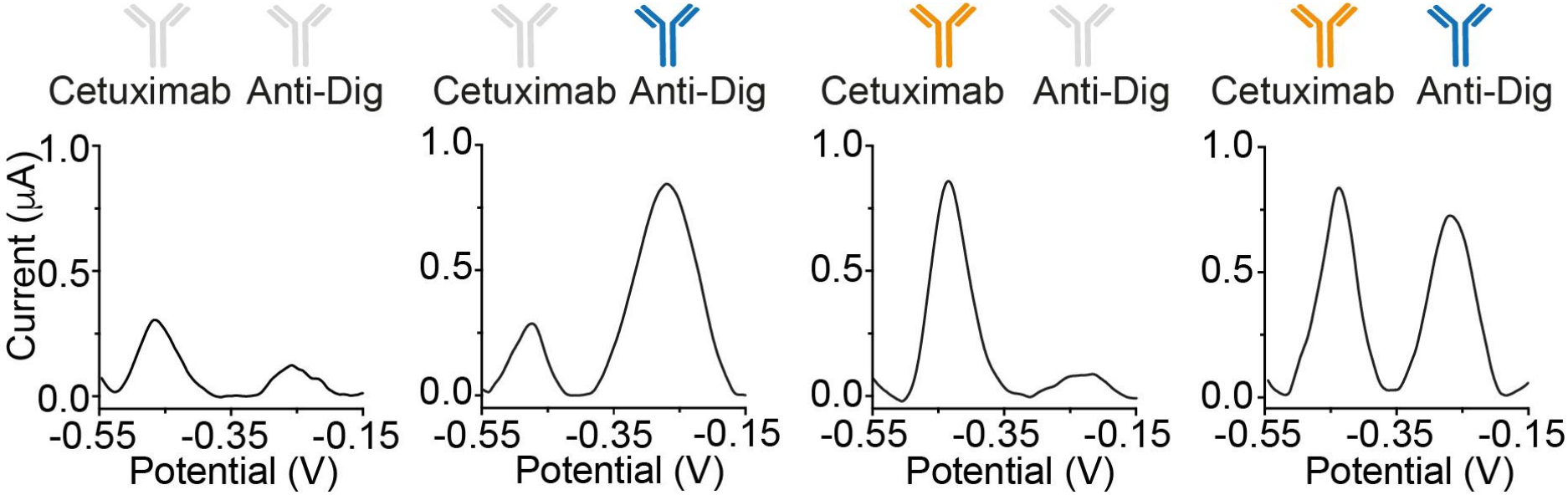



+Cetuximab
-Ab
Current (μA)
Potential (V)
[Cetuximab Antibody] (M)
Signal gain (%)
Cetuximab
Anti-Dig
Anti-DNP
Anti-HIV
Anti-HA
+Anti-HIV
[Anti-HIV Antibody] (M)
+Anti-HA
[Anti-HA Antibody] (M)

# Orthogonal platform: multiplexed antibodies detection



# Electrochemical DNA-based platform for antibody detection